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EFFECT OF HIGH TEMPERATURE ON THE LARVAL DEVELOPMENT OF *HYMENOLEPIS NANA* AND *HYMENOLEPIS DIMINUTA* (CESTODA: CYCLOPHYLLIDEA)*

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The different types of malformation and the variability in size seen in cysticercoids of *Hymenolepis nana* and *H. diminuta* grown at 30° C (Voge and Turner, 1956; Voge and Heyneman, 1957) stimulated interest in studies on the effect of higher temperature upon larval development of these cestodes.

The purpose of the experiments described below was to investigate the types of structural abnormalities produced in each species; what types of abnormalities would occur in both; whether or not structural abnormality could be related to infectivity of the larva in the definitive host; and what effect, if any, different temperatures had on patterns of development and ultimate size of the cysticercoids.

MATERIALS AND METHODS

Animals used in this study included the Rice Institute strains of *Hymenolepis nana* and *H. diminuta*, and laboratory-raised flour beetles, *Tribolium confusum*. For infectivity experiments, Wistar laboratory rats and the Heston A strain of white male mice, from the Cancer Laboratories, University of California, Berkeley, were employed. Beetles were infected as described by Voge and Heyneman (1957). In order to obtain greater uniformity in the developmental stages of the larvae, beetles were allowed to feed at 30° C for 6 hours only, then transferred to clean dishes with food and exposed to the desired temperature. In each experiment, controls were kept at 30° C or 32° C. Beetles were dissected in Ringer's solution. Cysticercoids were fixed in Bouin's fluid for 3 hours, and after repeated washing in alcohol, stained for 2 hours in a dilute aqueous solution of Ehrlich's haematoxylin. All measurements were obtained from stained specimens. In order to correlate results obtained at high temperature in the beetle host with known differences between the 2 species of tapeworms in adaptation to their vertebrate hosts, deep body temperatures of adult mice and rats were obtained using a small-bore thermometer graduated in units of 0.2° C. All temperatures were obtained at the same time of day. Thermometer readings were made 30 seconds after insertion.

RESULTS

Hymenolepis diminuta

Voge and Turner (1956) established that infective cysticercoids may develop at temperatures ranging from 15–37° C, and that development is abnormal or inhibited at 40° C. Abnormal development as well as reduction in body size were observed in some larvae grown at 37° C. Further information on the extent and nature of variability in body-size found in cysticercoids grown at different temperatures is presented in Table I. It will be noted that the only dimension which varies

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TABLE I. *Size of* Hymenolepis diminuta *cysticercoids grown at different temperatures.*

Temperature	Time	Measurements no. taken	Body Width μ	Body Length* μ	Scolex Width μ	Tail Length μ
20°C	1 month	16	153–348 243.9	306–391 344.2	78–160 98	28– 434 224
20°C	2 months	12	255–323 279.1	331–391 359.1	60–102 80.4	150– 743 512.6
25°C	14 days	27	162–247 199.2	255–315 290.3	60– 85 77.1	340–1020 543.1
30°C	8 days	73	144–289 198.3	204–467 300.1	76–160 84.8	170– 850 399.3
30°C	43 days	19	162–272 208.4	297–425 383.3	74– 90 81.6	221–1300 642.6
37°C	6 days	23	180–229 205.6	229–357 310.8	84–112 95.5	0– 306 141.9
37°C	8 days	27	172–280 203.2	221–357 293.6	68–108 82.7	0– 553 238.7

* Exclusive of tail.

markedly with temperature is that of the tail. While tail length may be as much as 1 mm or more in cysticercoids developed at 20° C, it is less than 0.5 mm at 37° C, or the tail may be absent entirely. However, as noted previously (Voge and Heyneman, 1957), the size of the scolex does not seem to be affected by differences in environmental temperature. Since the cysticercoid tail continues to grow after development of the larva has been completed, it was thought that the period of growth, rather than the temperature at which growth proceeds, may be responsible for these size differences. Comparison of ranges and averages of tail lengths in larvae grown for 8 days at 30° C, with those grown for the same time at 37° C, indicates that time alone is probably not responsible for the differences noted. At 37° C, development of cysticercoids is completed in 5 to 6 days; at 30° C, in 8 days. Structural development is completed 2 days sooner at 37° C than it is at 30° C. However, even if the larvae grown at 37° C are kept for the full 8 days necessary for development at 30° C, tail length in the 37° C larvae is smaller than it is in the 30° C larvae. It appears probable, therefore, that temperature, independent of time, affects size of the tail.

The normal development of *H. diminuta* at 30° C, has been described by Voge and Heyneman (1957). When grown at 37° C, certain structural characteristics and abnormalities appear. In comparison with controls developed at 30° C, 3 general effects are observable at 37° C. The first is the incomplete development or lack of the outermost membrane (Plate I, 1, 2) which usually envelops the cysticercoid exclusive of the tail portion. The second effect is on the cysticercoid tail which may be absent (Plate I, 4, 6) or short and twisted (Plate I, 5). Third, and most interesting, is the inhibition of scolex withdrawal in larvae otherwise normal in appearance. (Although the terms "invagination" and "withdrawal" are interchangeable, we prefer to use "withdrawal" as "invagination" has been used

TABLE II. *Incidence of normal and abnormal development in cysticercoids of* H. diminuta *grown at different temperatures.*

Temperature	Time in days	No. examined	Withdrawn	Not withdrawn, otherwise normal	Not withdrawn, abnormal
30°C (control)	8	73	73	0	0
37°C	5	25	14	11	0
37°C	7–8	37	23	14	0
38.5°C	5	28	1	9	18
38.5°C	8	23	1	6	16

for different processes. Table II shows some results obtained at 37° C. It should be noted that after 8 days at 30° C, all larvae had withdrawn, while of those grown at 37° C, only two-thirds or less had withdrawn. The proportion of withdrawn to non-withdrawn larvae may vary considerably in different experiments, but failure of scolex withdrawal was always observed in at least some individuals grown at 37° C. As far as could be determined, this failure is not associated with any morphological abnormalities. These larvae after 8 days have a well developed cavity, a normal scolex (Plate I, 3), and are indistinguishable from comparable stages seen after 5 or 6 days at 30° C.

Larvae grown at 38.5° C were either abnormal or failed to complete development. Of all larvae grown at this temperature, only 2 individuals had a withdrawn scolex (Plate I, 7, 8). In one of these, scolex withdrawal had occurred prematurely, before sucker differentiation had been initiated. In a third specimen (Pl. II, 1), scolex withdrawal had begun but the cavity was poorly delineated and the suckers barely distinguishable. A relatively small proportion of larvae appeared structurally normal, having a well developed cavity and scolex, but had failed to withdraw even after 8 days (Plate II, 2). Data from some of these experiments are shown in Table II. Comparison of results obtained at 38.5° C with those at 37° and 30° C, clearly demonstrates that here also temperature and not time is the critical factor inhibiting withdrawal.

Common malformations of larvae grown at 38.5° C included absence of a cavity (Plate II, 3, 5, 7), failure of sucker differentiation (Plate II, 4, 6, 8), and marked distortion of the body (Plate II, 4, 6, 8). To summarize: the general effects at 38.5° C are inhibition of growth, inhibition of structural differentiation, and failure to complete development (withdrawal) in those instances where structural differentiation appears to be normal.

Comparison of the infectivity of structurally normal cysticercoids developed at 30° and 37° C was made by feeding laboratory rats single cysticercoids. The results are shown in Table III. While the rate of infectivity was the same for

TABLE III. *Infectivity of cysticercoids of* H. diminuta *grown at 30° and 37° C.*

°C	Time in days	Rats fed	Rats infected
30	8	30	18
37	8	30	17
37	6	27	4

cysticercoids kept for 8 days at either 30° or 37° C, it was much lower in cysticercoids kept at 37° C for only 6 days. Yet, infective cysticercoids may occasionally be developed in as short a time as 5 days at 37° C (Voge and Turner, 1956), and these cysticercoids do not seem to differ structurally from those kept for either 6 or 8 days at this temperature. The reasons for this relatively low rate of infectivity after 6 days at 37° C are not apparent, and do not seem to be related to the absence of the well developed outer membrane seen in cysticercoids grown at 30° C.

In relatively heavy infections of the intermediate host the effects of high temperature are enhanced, so that the incidence of tailless or structurally abnormal larvae is higher than is usually seen in light infections. Such crowding effects may be observed when individual beetles harbor more than 12–15 cysticercoids. Of 40 cysticercoids obtained from 2 beetles incubated at 37° C, 21 had little or no tail, 9 had not withdrawn, and 8 were structurally abnormal.

Hymenolepis nana

The effect of different temperatures on normal larval development of *Hymenolepis nana* has been studied by Heyneman (in press) who found that this species can develop normally at temperatures considerably higher than *H. diminuta*. The general effect of high temperatures on size and structure of the larvae is similar to that observed in *H. diminuta*. Comparison of data in Table IV shows that the

TABLE IV. *Size of* Hymenolepis nana *cysticercoids grown at different temperatures.*

Temperature	Time	No. of measurements	Body Width (microns)	Body Length* (microns)	Scolex Width (microns)	Tail Length (microns)
20°C	14 days	81	98–160 127.4	116–198 150.7	70–110 89.2	72–198 *110.3*
25°C	13 days	22	120–156 135.6	120–196 158.6	80–112 95.8	74–272 *123.5*
30°C	6 days	54	106–182 144.2	108–196 158.6	72–130 96	0–246 *108.5*
30°C	18 days	50	100–180 139.2	116–200 159.2	68–122 92.7	70–340 *121.6*
37°C	4½ days	46	92–152 120.8	108–170 146.4	61–110 92.9	14–126 *53.9*
40°C	4 days	45	78–158 117.8	82–134 106.2	64–122 87.6	12– 98 *37.4*
40°C	7 days	48	88–190 132.1	108–220 166.7	78–124 98	0–150 *32.5*
42°C	7 days	17	94–130 122.2	120–182 144.7	84–110 95.4	0– 68 *30.3*

* Exclusive of tail.

structure chiefly affected by temperature is the tail. Length of the tail was much reduced in cysticercoids grown at 37° to 42° C for 4 to 7 days, and in many individuals no tail was present. Controls grown at 30° C usually had a well developed tail, as did cysticercoids grown at lower temperatures. The tail of *H. nana*, like that of *H. diminuta* continued to grow for some time after structural differentiation of the cysticercoid was completed. Seven-months-old cysticercoids had tails averaging 158 microns in length, while the highest average obtained in other age groups was 123 microns.

The effects of high temperatures upon structural differentiation and development of the cysticercoid differ somewhat from those observed in *H. diminuta*. Inhibition of scolex withdrawal, seen so frequently in *H. diminuta*, was rarely observed in *H. nana* raised at high temperatures. *H. nana*, in contrast to *H. diminuta*, normally withdraws the scolex very early in relation to differentiation of sucker and rostellum, and this may explain some of the differences noted between the reactions of the 2 species to high temperatures. The most frequent abnormalities seen in *H. nana* involved body shape and size, thickness and form of the enveloping membranes, and position and structure of the scolex. Plate III, 2, illustrates in general outline a normal cysticercoid of *H. nana* with well developed scolex and membranes, grown at 39° C. The slight twisting of the scolex also may occur at lower temperatures and is apparently not associated with infectivity. Figures 3 and 5, Plate III, show abnormal individuals obtained at the same temperature. Membranes and sucker musculature are absent, there is no cavity (Fig. 5), body size is reduced, and antero-posterior orientation is obscured. However, comparison with figure 7, Plate III, suggests that the narrower end is anterior. At high temperature, an abnormal amount of growth is seen to occur anteriorly, while tail growth may be completely inhibited. Plate III, 4, illustrates a larva obtained at 37° C, which looks relatively normal but lacks the thick outer membrane, as well as a tail. This type is referred to as "egg-shaped" in Table V. While de-

velopment at 37°C is usually normal, abnormalities in cysticercoid shape and structure of the membrane may occur (Plate III, 1). At 40°C and 42°C, most individuals are abnormal. Plate III, 6, and Plate IV, 1, 2, 3, 4, 5, illustrate various malformations, such as irregularities in body shape and in position of the scolex, irregular membrane development, incomplete differentiation of sucker musculature and an apparent lack of antero-posterior orientation. Complete failure of structural differentiation is illustrated in Plate IV, 6.

Data on incidence of abnormal development at different temperatures are shown in Table V. It is apparent that the number of abnormal individuals increases with

TABLE V. *Incidence of abnormal development in cysticercoids of* H. nana *grown at different temperatures.*

Temperature	Time in days	Nos. examined	Normal (with tail)	Egg shaped (no tail)	Completely abnormal
30°C (control)	6	48	47 (98%)	0	1 (2%)
37°C	4½	54	43 (80%)	7 (13%)	4 (7%)
39°C	4½	86	51 (59%)	18 (21%)	17 (20%)
39°C	7	69	41 (59%)	19 (28%)	9 (13%)
40°C	4½	29	16 (55%)	9 (31%)	4 (14%)
40°C	7	42	12 (29%)	21 (50%)	9 (21%)
40°C	14	26	5 (19%)	3 (12%)	18 (69%)
42°C	7	24	0	14 (58%)	10 (42%)

higher temperature. Results of experiments conducted at 40°C suggest that at this temperature the period of exposure may also be important, since more abnormal individuals are seen after 14 days than after 4½ or 7 days. The increase in number of tailless larvae at higher temperatures has already been noted.

Infectivity of cysticercoids grown at temperatures ranging from 32° to 42°C, was tested in mice fed with a single larva each. The results of these experiments are presented in Table VI, which shows a sharp lowering of infectivity of cysticer-

TABLE VI. *Infectivity of single cysticercoids of* H. nana *grown at different temperatures.*

Temperature °C	Time in days	Mice fed	Mice infected
32	5	53	34
37	4	15	12
39	4	12	5
39.5	4	18	0
39.5	19	10	1
41.5	7	23	0
41.5	13	10	0

coids grown at 39°C or higher temperatures. No infection was produced from feeding larvae grown above 39.5°C. Eighteen mice fed single cysticercoids which were developed for 4 days at 39.5°C had no infection at autopsy 12 days later, but 1 of 10 mice fed 19-day-old cysticercoids developed at this temperature did become infected. In addition, 4 of 12 mice each fed 10 of the same group of 19-day-old, 39.5°C cysticercoids, became infected. At autopsy, these infected mice had 1, 2, 3 and 7 worms. A single mouse fed 43 of these cysticeroids had 21 worms at autopsy.

In view of the greater tolerance of *H. nana* to high temperatures, and because

of the ability of this species to complete larval development within the mammalian host, it was of interest to obtain deep body temperatures of the rodent hosts and to compare the morphology of cysticercoids from mammals with those developed in beetles maintained at the same temperature. In 21 adult male mice, body temperatures ranged from 36° to 39.6°C, with an average of 38.3°C; in 10 female mice it ranged from 36.2° to 38.4°C, averaging 37.7°C. Ten adult female rats had body temperatures ranging from 36.8° to 38.2°C, averaging 37.7°C. In 10 adult males the range was 37.4° – 38.8°C with an average of 38.0°C.

DISCUSSION

The data presented in this study suggest that high temperature affects larval development of both *H. nana* and *H. diminuta* unfavorably. The commonest effect is a marked reduction of tail size and of the developing membranes. Tail, scolex and inner membrane contain large amounts of polysaccharide (Heyneman and Voge, 1957), but it is not known whether a reduction of this energy reserve is correlated with the changes seen at high temperature. Polysaccharide is still present in the scolex, even in the tailless forms.

In *Hymenolepis diminuta* normal development does not occur at 38.5°C, while a large proportion of normal infective larvae is obtained at 37°C. In *H. nana*, a marked retardation of development occurs between 39.5°C and 40.5°C. At the latter temperature development is delayed by 3 days in comparison with that at 39.5°C (Heyneman, in press). A marked change in infectivity also occurs. In the present study, no infection of mice was obtained with cysticercoids developed above 39.5°C. Yet, the fact that in *H. nana* development can occur at a temperature as high as 42°C, is in marked contrast with *H. diminuta* in which completion of development is inhibited at 38.5°C.

The differences in response between *H. nana* and *H. diminuta* to the same experimental conditions are striking. *H. nana* has a greater temperature tolerance than does *H. diminuta*, both with respect to maximal temperature at which development can occur and with regard to differences in infectivity in the final host. In both species, the transition from a relatively favorable to a definitely inhibitory environmental temperature appears to occur abruptly. *H. nana* cysticercoids, unlike those of *H. diminuta*, are not affected with respect to withdrawal of the scolex into the cysticercoid cavity. Our observations suggest a more precocious withdrawal for *H. nana* larvae at higher than at lower temperatures. As noted earlier, the scolex of *H. diminuta* is more fully developed at the time of withdrawal than is that of *H. nana*. Preliminary experiments with *H. diminuta* indicate that withdrawal can be inhibited by exposing developing larvae to high temperatures some time *before* withdrawal would normally occur. It therefore appears that in *H. diminuta* inhibition occurs early, but does not become apparent until the organism is further developed.

Also of interest is the difference between the capacity for structural development and infectivity of larvae of *H. nana*. A fully formed scolex may be obtained at 42°C; yet no infection has yet been obtained with larvae grown above 40°C. This serves to emphasize the importance of distinguishing between structural development and infectivity. As observed previously (Heyneman, in press) the failure of the fragile, thin-walled cysticercoids developed at 41° to 42°C to infect the mouse may be simply the result of inadequate protection from the host's stomach

acids. Yet, cysticercoids developed at 37° and 39°C can pass this barrier successfully, despite the absence of well developed membranes. Likewise, a large proportion of *H. diminuta* larvae kept for 8 days at 37°C, are infective and yet do not possess the well developed outer membranes seen in cysticercoids developed at lower temperatures.

Measurements of deep body temperatures of the rat and the mouse hosts show 2 features pertinent to this discussion. First is the surprisingly high individual variation in body temperature; second, body temperature, in some individuals at least, is high enough to prevent development of *H. diminuta* but not of *H. nana* cysticercoids. However, even for *H. nana*, mouse temperatures might be sufficiently high to retard or to inhibit development of a large proportion of larvae. Evidence for this is seen in the striking similarity between cysticercoids taken from the villi of mice shown in Brumpt (1949, Fig. 513, p. 782) and those illustrated in the present study, grown in beetles at corresponding temperatures. Reduction in total cysticercoid size, the loss of membranes, polar swellings, and lack of tail, are characteristic both of our high temperature "abnormals" and of larvae illustrated by Brumpt as *H. nana* var. *fraterna*.

It thus appears that the mouse is perhaps only a partially successful host for the larvae of *Hymenolepis nana*, if the variety of cysticercoid shapes formed is related to subsequent establishment of the young worms in the intestine. It also seems reasonable to assume that body temperature of the warm-blooded host is at least one of the critical factors preventing *H. diminuta* from undergoing larval development in the vertebrate. It appears, furthermore, that the development of a high temperature tolerance in *H. nana* is not complete and that this species has not yet become fully adapted to the warm-blooded host.

Studies are in progress to determine the loss of larvae of *H. nana* between the time of development of cysticercoids in the villi and the establishment of young worms in the intestinal lumen. Such studies may help to explain the relatively low rate of infectivity of cysticercoids developed in beetles at temperatures corresponding to those of the warm-blooded host.

SUMMARY

The rat tapeworm, *Hymenolepis diminuta*, develops in the intermediate host, *Tribolium confusum*, to a mature and infective cysticercoid larva at environmental temperatures as high as 37°C. However, cysticercoid structure is markedly affected at this temperature. The tail is dwarfed or absent, and the outer membrane absent or poorly developed. Completion of structural development at 37°C occurs after 5 days, but does not necessarily coincide with infectivity. At 38.5°C, *H. diminuta* is not able to complete development. The larvae are either abnormal or fail to withdraw the scolex into the cysticercoid cavity, regardless of additional time allowed for completion of development.

Larvae of *Hymenolepis nana*, the dwarf tapeworm of rodents and man, have a greater tolerance for high temperatures than do the larvae of *H. diminuta*. Development may proceed at a temperature as high as 42°C. Failure of scolex withdrawal was observed very rarely. As in *H. diminuta*, the larval tail and membranes are much reduced or absent. Other changes include asymmetry of the cysticercoid with abnormal distribution of some component parts, and extreme fragility and transparency of the larva.

Infectivity of *H. nana* in the mouse host is affected at a lower temperature than is structural development. No infection was obtained from cysticercoids which had developed above 39.5°C; yet complete structural development may proceed at 42°C. Larvae obtained from beetles kept at temperatures ranging from 39° – 41°C, resemble those recovered from the villi of mice. Since deep body temperatures of mice may be as high as 39.6°C, temperature may be largely responsible for these similarities in morphology.

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Explanation of Plates

All figures drawn with the aid of a camera lucida. Drawings emphasize abnormal stages. For illustrations of normal developing and mature cysticercoids see Voge and Heyneman, 1957.

Hymenolepis diminuta

Figure 1. Structurally normal cysticercoid but lacking external enveloping membrane; 37° C, 5 days.

Figure 2. Cysticercoid as in figure 1; 37° C, 7 days.

Figure 3. Scolex withdrawal inhibited; 37° C, 5 days.

Figure 4. Cysticercoid lacking external membrane and tail; 37° C, 8 days.

Figure 5. Cysticercoid with two tail buds. External membrane absent; 37° C, 8 days.

Figure 6. Scolex normal, membranes and closure of cavity incomplete; 37° C, 8 days.

Figure 7. Scolex withdrawal without sucker differentiation; 38.5° C, 5 days.

Figure 8. Scolex withdrawal with incomplete sucker differentiation. Tail and external membrane absent; 38.5° C, 8 days.

Hymenolepis diminuta

Figure 1. Partial withdrawal of scolex. Suckers barely observable; 38.5° C, 5 days.

Figure 2. Failure of scolex withdrawal in structurally normal larva; 38.5° C, 5 days.

Figures 3, 4, 5, 6, 7. Various abnormal stages showing irregularities in size, proportions, and lack of structural differentiation; 38.5° C, 5 days.

Figure 8. Abnormal larva with suckers differentiated but without cavity; 38.5° C, 8 days.

Hymenolepis nana

Figure 1. Cysticercoid showing abnormalities in size, membrane development and position of scolex; 37° C, 8 days.

Figure 2. Normal larva except for twisted scolex and relatively small tail; similar to some observed at 30° C; 39° C, 7½ days.

Figure 3. Abnormal larva without tail or membranes and with abnormal growth anteriorly; 39° C, 7 days.

Figure 4. "Egg-shaped" larva without tail. Membrane development incomplete, rostellum twisted; 37° C, 4½ days.

Figure 5. Abnormal larva from a beetle which harbored 110 cysticercoids; 39° C, 4 days.

Figures 6, 7. Abnormal larvae; 40° C, 7 days.

Hymenolepis nana

Figure 1. Abnormal larva; 40° C, 14 days.

Figures 2, 3, 4. Various abnormalities; 40° C, 7 days.

Figure 5. Larva with relatively normal scolex; 42° C, 7 days.

Figure 6. Undifferentiated stage; 40° C, 7 days.

PLATE I

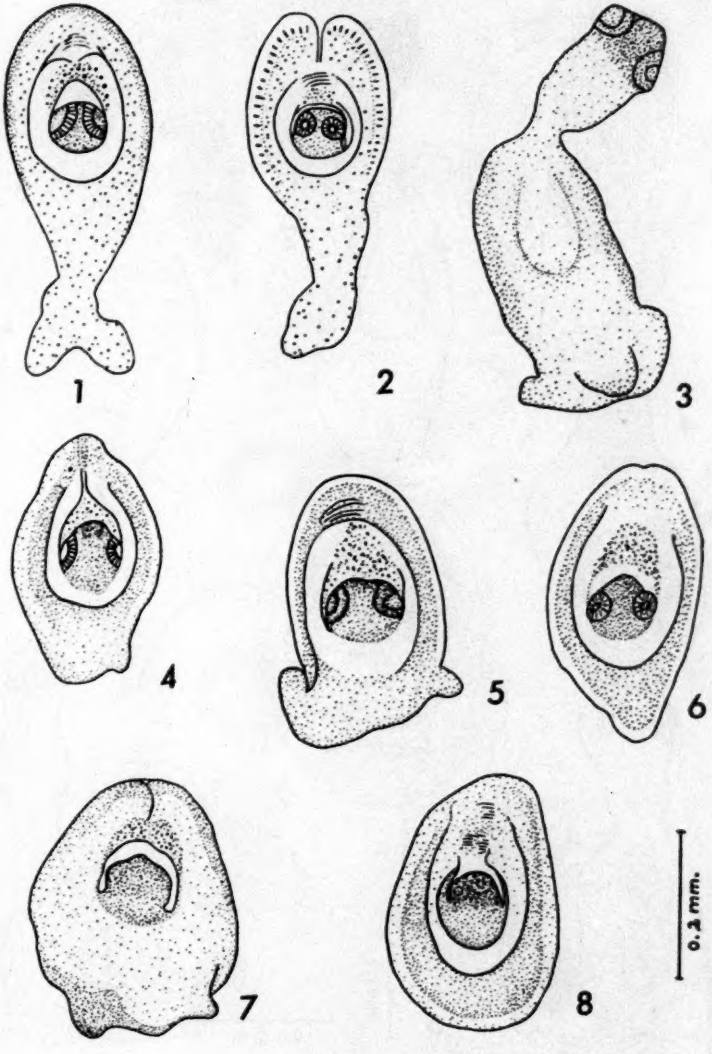


PLATE II

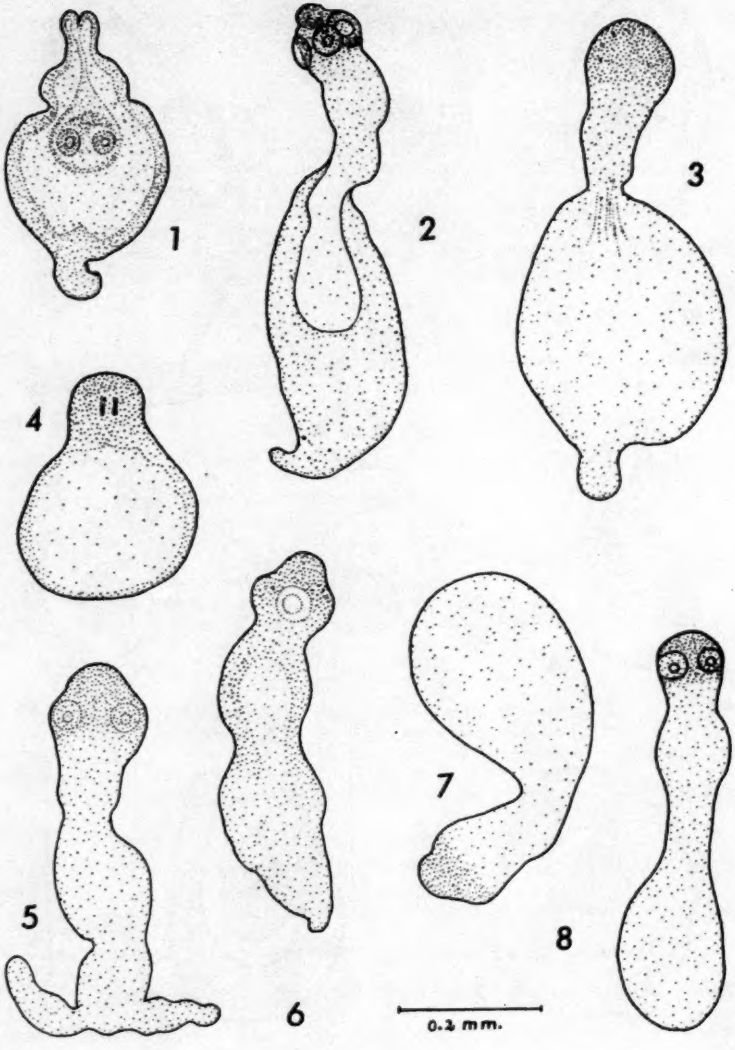


PLATE III

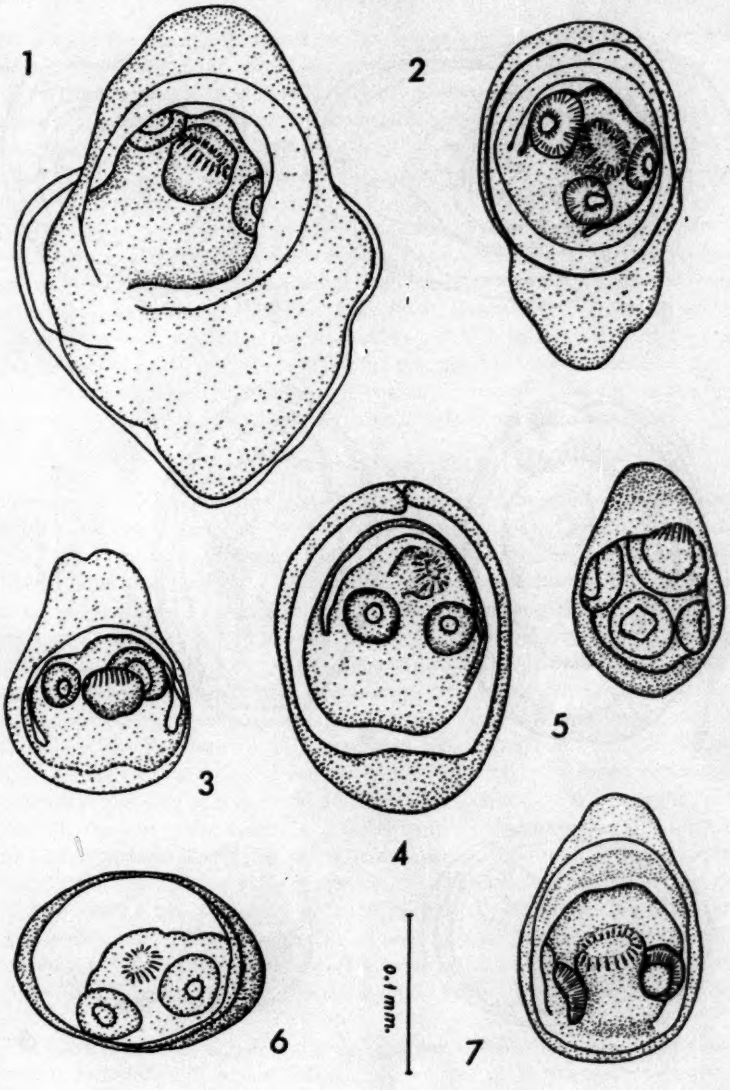
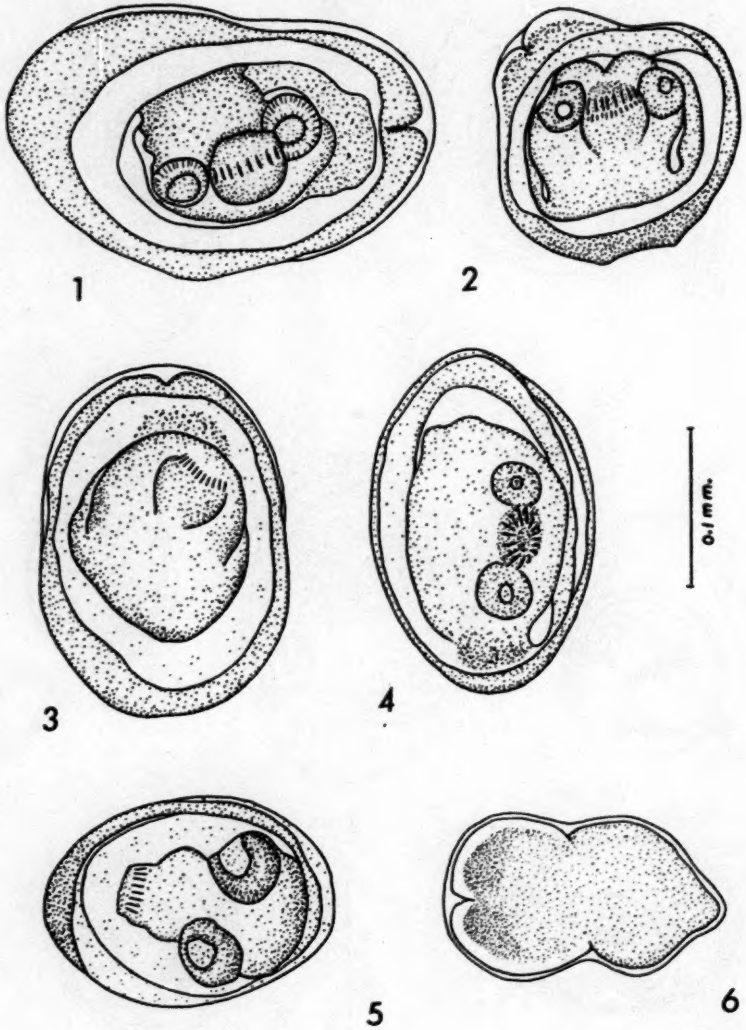


PLATE IV



THE TAXONOMY OF NEMATOTAENIID CESTODES

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At present the taxonomic status of the Nematotaeniidae as well as the species included in the family is unsettled. Lopez-Neyra (1944b) placed this group in the Hymenolepididae, giving it subfamily status. Reviews since 1944 (Hyman, 1951; Wardle and McLeod, 1952) have necessarily been brief and do not refer to Lopez-Neyra's paper. Also, no recent review of the family contains a complete list of species, and the taxonomic relationships of species have not been discussed in any detail since 1935 (Hsü, 1935).

In addition to a taxonomic review the present paper includes descriptions of 2 species. One was taken from *Hyla regilla* Baird and Girard, in the Willamette River valley of Oregon, and the other from *Batrachoseps attenuatus attenuatus* (Eschscholtz), in and near Berkeley, California. Forty-three *H. regilla* and over 200 specimens of *B. a. attenuatus* were examined for parasites. Cestodes, when present, occurred in the anterior portion of the small intestine. Because the incidence of infection and number of worms per host is complicated by variation with the seasons, a detailed account of such matters is deferred to a later paper.

TAXONOMY OF THE NEMATOTAENIIDAE

Cestodes which will here be considered as properly belonging to the Nematotaeniidae have been placed in the following families: (1) Taeniidae, by Ludwig (1886), (2) Hymenolepididae by Ransom (1909) and by Lopez-Neyra (1944b), (3) Dilepididae by Fuhrmann (1907, 1908), and (4) Nematotaeniidae, Lühe, 1910. Since the present day Taeniidae is much less encompassing than Ludwig's family, it does not seem necessary to present evidence that nematotaeniids do not belong to that family. The remainder of the dispositions listed deserve more attention.

In 1907 Fuhrman established a new family, the Dilepinidae (changed to Dilepididae by Railliet and Henry in 1909), and placed *Nematotaenia dispar* in 1 of its 3 subfamilies, the Paruterinae. He explained this disposition of *N. dispar* on the basis of the developmental and morphological affinities of its paruterine organs with the corresponding organs of the Paruterinae. Jewell (1916), however, pointed out that the known nematotaeniids differed considerably from the other members of the Paruterinae in possessing more than 1 paruterine organ per proglottid and in forming uterine capsules early in uterine development. Because of these differences she erected a new subfamily of the Dilepinidae for the reception of the known nematotaeniids, the Cylindrotaenianae. Poche (1925) indicated that Jewell was unaware of Lühe's (1910) creation of a separate family (Nematotaeniidae) for *N. dispar* as well as Railliet and Henry's (1909) action of renaming of the Dilepinidae.

The placement of *N. dispar* in the Dilepididae is difficult to justify on the basis of present knowledge of nematotaeniids. In terms of paruterine development it appears that the nematotaeniids are just as closely related to the Davaineidae or the Anoplocephalidae as they are to the Dilepididae (see Fuhrmann, 1931 for the concepts of families referred to in this paper). Indeed, the writer agrees with Poche

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(1925) that, if anything, they are more closely related to the anoplocephalids than to the dilepidids. (A rostellum or a rudiment thereof is always present in the Dilepididae while it is always absent in the Anoplocephalidae and Nematotaeniidae.) In referring to nematotaeniids neither Fuhrmann (1907) nor Jewell (1916) mentioned other families which include members possessing paruterine organs. For Jewell, this is understandable in the case of the Anoplocephalidae (subfamily Thysanosominae), since Gough (1911), in a monograph dealing with the genus, *Stilesia* and a histological study of *Avitellina centripunctata*, concluded that the paruterine organs of these forms are derived from the uterine wall. Therefore one might conclude that the uterine-derived paruterine organ in *Avitellina* is not homologous to parenchyma-derived organs of *Metroliasthes* (family Dilepididae) and *Cylindrotaenia*. Jewell, 1916. However, the figures of Gough do not indicate anything appreciably different in development and morphology of paruterine organs from that occurring in *Metroliasthes* or *Baerietta*. Similarly, Hsü (1935, Figs. 41, 42) and Jewell (1916, Figs. 2, 3, 4) have figured paruterine organ formation in *Cylindrotaenia*, and Dickey (1921) stated that the paruterine organs arise from parenchyma adjacent to the uterus. In *Mesocestoides* most authors are agreed that the paruterine organs (= "egg capsules") arise from specialized cells, associated with the posterior end of the uterus, and not from the uterine wall itself (see Hamann, 1885; Hoeppli, 1925; Byrd and Ward, 1943; and Chandler, 1946 and 1947). Byrd and Ward have referred to these cells as paruterine cells. The Idiogeninae (family Davaineidae) also possess paruterine organs which are quite similar to those occurring in the Nematotaeniidae (Clausen, 1915). Other characters which might relate the group in question to either the Anoplocephalidae, Davaineidae, or Dilepididae seem to be lacking or insignificant; and, unless drastic changes are made (such as erecting a new family for all forms with paruterine organs), the nematotaeniids must be excluded from all three.

The nematotaeniids have been placed in the Hymenolepididae on 2 separate occasions. *N. dispar* was placed in the Hymenolepididae by Ransom (1909) when he combined Fuhrmann's (1907) Hymenolepididae and Dilepinidae (= Dilepididae) into one family; and, more recently, Lopez-Neyra (1944b) referred the described nematotaeniids to the Hymenolepididae as a subfamily (the Nematotaeniinae). Ransom did not justify his action but Lopez-Neyra's was based on 4 traits which were possessed by both the hymenolepids and nematotaeniids. These are: (1) a small, definite number of testes, (2) sex ducts (vagina and cirrus) which were dorsal to both excretory canals, (3) an external seminal vesicle (in *Baerietta* and *Cylindrotaenia*) and (4) a sac-like or horseshoe-shaped uterus. Superficially this appears to be a significant number of categories to justify the consolidation of the 2 families, but close examination of each category will reveal the difficulties involved in such a union. Mayhew (1925) and Voge (1952), in studying testicular lobation and complex branching of vasa efferentia in the Hymenolepididae, have found evidence that each testis of that family is a compound structure, being phylogenetically derived from several simple testes. On the other hand (with the exception of species intermediate between *Baerietta baeri* and *Cylindrotaenia*—see page 268), there are no indications that the testes in the Nematotaeniidae are compound structures. Furthermore, the presence of a limited number of testes is not peculiar to these 2 families but occurs also in the Dilepididae, where 4 testes occur regularly in *Paradilepis duboisi* Hsü, 1935. The position of the sex ducts in relation to the

excretory canals might be thought to be of considerable importance since basic differences in embryological symmetry are implied by such a relationship. However, many families are not at all uniform with respect to these characters (e.g., Baer, 1927) and even among the Hymenolepididae there occur at least 3 species (*Hymenolepis asymmetrical* Fuhrmann, 1918; *H. spinosa* von Linstow, 1906; and *H. clausa* von Linstow, 1906) whose sex ducts are regularly located dorsal to or between the excretory canals. An external seminal vesicle (i.e., one not within the cirrus sac) occurs irregularly throughout the Cyclophyllidea (except in the Hymenolepididae where it occurs regularly); and, since an external seminal vesicle does not occur in all the nematotaeniids, it is not unlikely that its appearance in *Cylindrotaenia* and *Baerietta* is a special case. There is also a possibility of misinterpreting a minor swelling in the vas deferens as a seminal vesicle. Such a swelling was noted in *Baerietta diana* (Fig. I b), but it was so slight that the writer did not designate it as a seminal vesicle. A sac-like uterus is most likely a generalized type of organ in that it occurs irregularly in many families and, therefore, is probably not distinctive enough to be a family characteristic. This does not discount the value of utilizing uterine morphology in those instances where the uterus assumes rather specialized characteristics as in the Taeniidae. No instance of a distinctly horseshoe-shaped uterus occurring in the Hymenolepididae has come to the attention of the writer although it is well illustrated in the Davaineidae and in the Dilepididae (*Paruterina candelabraria* (Goeze, 1782) and *Biuterina paradisea* Fuhrmann, 1902). Further, there are many exceptions to the occurrence of a sac-shaped uterus in the Hymenolepididae. For instance the uterus in the genus *Hymenolepis* is often distinctly lobed, as in *H. lobulata* Mayhew, 1925, *H. kempi* (Southwell, 1921), *H. sacciperium* Mayhew, 1925, and *H. lateralis* (Mayhew, 1925); uteri in the same genus may also be sheet-like lobed organs weakly convoluted so as to partially encircle the other reproductive organs (*H. macrostrobilodes* (Mayhew, 1925) and *H. fryei* (Mayhew, 1925)); or they may even be reticulate as is characteristic for the entire genus *Fimbriaria* (Lopez-Neyra, 1944b, however, suggested placing this genus in a separate family). On the other hand, the typical uterus in the Nematotaeniidae is horseshoe-shaped.

Although, as related above, the 4 criteria given by Lopez-Neyra for incorporating the nematotaeniid group in the family Hymenolepididae are of rather dubious constancy, it may still be argued that in spite of these inconsistencies the number of criteria alone is sufficient for such a consolidation. However, the nature of these inconsistencies and the number of exceptions seems too great for the writer to affirm such a consolidation. This is especially true if one considers that the Hymenolepididae do not have paruterine organs and do not occur in amphibians.

The only remaining disposition to be considered is that of the recognition of a new family (Nematotaeniidae Lühe, 1910) for the reception of the group of organisms in question. Lühe's family is widely recognized by recent authors (Ward 1918, Poche 1925, Fuhrmann 1931, Hyman 1951, Wardle and McLeod 1952). Although further investigation is needed to reveal whether Lühe's action was justified, available evidence indicates that the Nematotaeniidae form a well circumscribed group. It is the only family which consistently exhibits all of the following 4 characters: (1) paruterine organs which develop at the anterior surface of the uterus, (2) a small but definite number of testes, (3) a cylindroid form, and (4) parasitism in either amphibians or reptiles. Indeed, no other family contains representa-

tives which exhibit any more than 2 of the above 4 characteristics simultaneously. The truth of this statement can be made obvious by examining the various families which exhibit at least 1 of the above traits. Only 4 genera (not nematotaeniids) possess 2 of the above 4 traits. *Paradilepis duboisi* Hsü, 1935 possesses 4 testes and a cylindroid form, while the genera *Stilesia*, *Avitellina*, and *Anonchotaenia* (Baer, 1927) have paruterine organs and a cylindroid form. Otherwise those families which contain genera possessing paruterine organs (Dilepididae, Anoplocephalidae, Mesocestoididae, Davaineidae) do not possess any of the other 3 characters listed above. The only families which contain members with a small number of testes are the Dilepididae, mentioned above, and the Hymenolepididae. The Hymenolepididae, again, do not exhibit any of the other 3 traits already listed. Among the Cyclophyllidea, the only adult parasites of amphibians are nematotaeniids, while only 1 other family (the Anoplocephalidae) has adult representatives among reptiles.

As mentioned above, future work may yield new evidence which will indicate strong affinities of the Nematotaeniidae with another family. But at present it appears to be as well, or better, defined than most families in the Cyclophyllidea.

NEMATOTAENIID GENERA AND SPECIES

The nematotaeniid cestodes described to the present include the following 4 genera: *Nematotaenia* Lühe, 1899; *Cylindrotaenia* Jewell, 1916; *Distoichometra* Dickey, 1921; and *Baerietta* Hsü, 1935.

Nematotaenia dispar (Goeze, 1782) Lühe, 1899 (see Hsü, 1935 for synonyms) was not adequately described until Fuhrmann (1895) provided a detailed account of its morphology. Fuhrmann's material came from *Salamandra atra* Laur., *Rana temporaria* L., and *Bufo vulgaris* Laur. He depicted a cylindrical cestode whose diameter decreases gradually from a thick anterior end, measuring 0.94 mm, to a slender posterior end, measuring 0.14 mm. Its length averages 140 mm, although worms as long as 220 mm were reported. The mature proglottid is round in cross section and includes 2 dorsally placed oval testes, a ventral placed spherical ovary, and a rounded vitelline gland slightly dorsal and medial to the ovary. Dispersed more or less irregularly in the parenchyma of the gravid proglottids were 13–30 paruterine organs, each of which contained 3 (rarely 2 or 4) onchospheres.

O. Schmitt (1855) reported a cestode in *Rana temporaria* L. which may have been *N. dispar*. The worm he described had a long slender neck which measured only 2/5 the diameter of the worm at the level of the fully formed testes. His description of egg capsule development parallels paruterine development in *N. dispar*.

Leidy (1851) provided a very brief description of a cestode which was probably a nematotaeniid. In comparing this cestode with *N. dispar* he came to the conclusion that it belonged to a separate species—*N. pulchella*. Although the present status of this cestode is not settled, it is probably not a *Nematotaenia* as this genus has not been reported from the western hemisphere in other papers.

The hosts of *N. dispar* were listed by von Linstow in 1899. (In 1899 *N. dispar* was the only nematotaeniid known; so it is surely not in all the hosts he listed.) These are: *Salamandra atra* Laur., *S. maculata* L., *Necturus maculosus* (Rafin.), *Rana temporaria* L., *R. pipiens* Gm., *Pelobates fuscus* Laur., *Bufo vulgaris* Laur., *B. americanus* Leconte, *B. viridis* Laur., *Hyla arborea* L. and *Platydactylus guttatus* Guerin. Stiles and Hassall (1912) added the following hosts of *N. dispar:*

Menobranchus maculatus (Rafin.), *Ascolobates mauritanicus* (L.), *Bufo cinereus* Schneid., *B. lentiginosus* Shaw, *Rana halecina.* Walton (1941) has cited the following hosts of *N. dispar* which were not included in either of the other host lists: *Bufo bufo* Steind., *Rana agilis* Thomas, *R. esculenta* L., *Salamandra salamandra* L. and *Triturus alpestris* Laur. all of which occur in Europe; *Bufo* sp.? and *B. melostictus* Schneid. of India (doubtful); *Acris gryllis* Gunth., and *B. terrestris* Rösel of the United States.

Lopez-Neyra (1944a) noted that specimens ascribed to *N. dispar* occurring in amphibians differed somewhat from those occurring in reptiles. He named those from reptiles *Nematotaenia tarentolae.* The hosts were *Tarentola mauritanica* L. and *Platydactylus guttatus* Guerin which occur in Spain, southern France, and Italy. *N. tarentolae* differs from *N. dispar* in the following respects: (1) the scolex has a lesser diameter (280–500 microns in *N. dispar* and 200 microns or less in *N. tarentolae*), (2) the suckers of *N. tarentolae* are smaller (a diameter of 110–200 microns in *N. dispar* and 78–100 microns × 60–75 microns in *N. tarentolae*), (3) the cirrus sac is smaller in *N. tarentolae,* measuring about 75–110 microns in length whereas the same organ measures approximately 270 microns in length in *N. dispar,* and (4) there is a tendency for each paruterine organ of *N. tarentolae* to contain 1 or 2 eggs while there are usually 3 eggs in each paruterine organ of *N. dispar.* Aside from these morphological differences, there is apparently a host-parasite specificity existing between the lizards mentioned and *N. tarentolae,* since frogs and salamanders (hosts normally harboring *N. dispar*) in the same environment as the lizards were not found infected with *N. tarentolae.*

Nematotaenia lopez-neyrae Soler, 1945 was described from Bufo sp. This form differs from *N. dispar* in size and proportions, in the possession of a spiny protrusible cirrus organ, and in having a more symmetrical placement of the paruterine organs (the bases toward the middle of the proglottid with the apical bulbs lateral).

The genus *Cylindrotaenia* is represented by 2 species, *C. americana* Jewell, 1916, and *C. quadrijugosa* Lawler, 1938. Jewell's description of *C. americana* reported a cylindrical white cestode. It averaged 25–40 mm in length, about 1/5 of which is neck. The diameter of the type ranges from 130-180 microns, the average being about 140 microns. The single dorsal testis, at its maximum development, is located about 1/4 the distance from the scolex to the posterior end. Mature proglottids contain a spherical ventral ovary just dorsal to which is the vitelline gland; proceeding dorsally there was described, in this order, an immature, growing uterus, a club-shaped cirrus sac, and a single dorsal testis, which has passed its maximum development. At an average of 22 mm posterior to the scolex, 2 paruterine organs, 1 dorsal and 1 ventral, are fully developed; and external segmentation is, for the first time, conspicuous. Each paruterine organ is comprised of 2 portions, a basal one and a bulbar apical one, and usually contains 3 embryos. The cuticle is 3-layered, being only 3–4 microns thick. Compared to the musculature of *N. dispar,* that of *C. americana* is quite weakly developed. The subcutaneous musculature is especially poorly developed and the dorso-ventral muscles are completely lacking.

Hsü (1935) has listed the following hosts of *C. americana: Acris gryllis* Günth., *Pseudacris triseriata* Wied., *Rana pipens* Gm. (probably *C. quadrijugosa*—see next paragraph), *R. virescens* Kalm, *Hyla squirella* Daud., *Bufo lentiginosus* Shaw, and

Leiolopisma laterale Say. Also Savazzini (1929) has reported the same worm in *Leptodactylus ocellatus* (L.) Gir., and Ingles (1936) has reported it in *Bufo canorus* Camp.

C. quadrijugosa Lawler, 1938, is quite similar to *C. americana* except in the following characteristics: (1) *C. quadrijugosa* is slightly larger in all dimensions, (2) the vas deferens, cirrus sac, and female organs are not enclosed in a delicate membrane as Jewell described for *C. americana,* (3) the cuticle is ridged in *C. quadrijugosa,* and (4) a duct is formed in *C. quadrijugosa* which connects the paruterine organs to the uterus (in *C. americana* the uterus breaks down into individual capsules surrounding each egg). This species was described from material collected in *Rana pipens* Gm.

Distoichometra bufonis Dickey, 1921, which occurs in *Bufo lentiginosus* Shaw is the only described species of the genus Distoichometra. The total length of the worm is about 100 mm, the average diameter 430–440 microns. The neck is very short, comprising only 1/30 the worm's total length. Proglottids are fully matured about 12 mm posterior to the scolex. The topography of the reproductive organs is essentially the same as in *N. dispar,* the major difference being in the number and arrangement of the paruterine organs, *D. bufonis* having 2 rows of them with 4 to 6 in each row. The paruterine organs develop quite early, being present in the anterior 1/4 of the worm.

One new species of Distoichometra is described in the present study. This description was based on measurements taken from 6 whole mounts and 8 sets of serial sections.

Distoichometra kozloffi nov. sp.
(Plate II, Figs. a–d)
(All measurements in microns unless otherwise indicated)

Diagnosis: Total length of adult worm 22–47 mm, averaging 31 mm; scolex not well defined, unarmed, without a rostellum, averaging 322 in diameter; suckers circular to subcircular with a diameter of 112–123; neck of relaxed specimens greater in diameter than the scolex, measuring 460–525 in diameter; mature and early gravid proglottids scarcely distinct externally with a diameter of 350–710 and lying about 7 mm posterior to the scolex; testes 2 in number, oval, measuring 96–124 dorsoventrally, and 44–63 laterally; ovary single, globate, mid-ventrally located, and averaging 76 in diameter; vitelline gland distal to the ovary, spheroidal, measuring 43–50; uterus distinctly to scarcely horseshoe shaped, or tubular, centrally located, quickly subdividing into embryo capsules, not distinctly delineated from surrounding parenchyma, lateral dimension averaging 320 while the anteroposterior dimension averages 60; paruterine organs 6 in number, paired, 3 dorsal and 3 ventral, consisting of stalked bulbular structures in advanced proglottids, each bulbular portion containing 2–5 eggs (commonly 3), and measuring 100–110 in diameter; advanced eggs (including the capsules) ovoid, measuring 31–61 in length and 19–41 in width; hexacanth embryo subspherical to ovoid ranging from 14×30 to 14×15; cuticle 9–11 thick, occasionally with very delicate protruding fibrils; calcareous bodies not present on surface of cuticle, although they are rarely located within the parenchyma.

Host: Hyla regilla Baird and Girard.
Locality: Willamette River Valley, Oregon.
Habitat: Small intestine.
Type: U. S. National Museum.

Discussion: The description supplied by Dickey (1921) for *Distoichometra bufonis,* except for size differences and differences in the number of paruterine organs, could very aptly be applied to the present species. They both have the same number of testes and both possess more than 2 dorsoventrally paired paruterine organs. It may well be that the present species is phylogenetically intermediate between the 2 genera *Baerietta* and *Distoichometra.* However, it is, for the present,

placed in the latter genus because of its closer similarity to the genus *Distoichometra*.

It will be necessary to modify the generic diagnosis for *Distoichometra* as follows in order for it to receive the present species:

Distoichometra Dickey, 1921 emended: with 2 testes per segment, paruterine organs 6 in number or numerous, each with 2 to 6 uterine capsules, with bases fused, arranged in 2 parallel rows. The genus contains 2 species: *D. bufonis*, the type species, occurring in *Bufo woodhousii fowleri* (Hinkley), *B. terrestris* (Bonnaterre) and *Scaphiopus* sp., and *D. kozloffi* occurring in *Hyla regilla* Baird and Girard.

Hilmy (1936) has suggested that the difference between cestodes placed in the genera *Distoichometra* and *Nematotaenia* is only specific since they are separated generically chiefly on the basis of the smaller number of paruterine organs in the former. However, other than difference in the numbers of paruterine organs, these 2 genera also differ in the orientation and arrangement of these organs. Those of *Distoichometra* are oriented with the apical bulb anterior and the basal portion posterior, and, in addition, the paruterine organs of this genus are arranged in 2 rows disposed dorso-ventrally to each other. In *Nematotaenia*, on the other hand, the paruterine organs are oriented (if at all regularly) with their basal regions toward the periphery of the proglottid, forming a kind of rosette. Since *Distoichometra* resembles *Baerietta* in arrangement and orientation of paruterine organs and, at the same time, resembles *Nematotaenia* in possessing an indefinite number, it seems that it should be considered as an intermediate genus between *Nematotaenia* and *Baerietta* and therefore not identical with either. For the same reason (*i.e.*, arrangement and orientation of the paruterine organs), as pointed out by Hsü (1935), *Baerietta* should be considered as a genus separate from *Nematotaenia*.

The genus *Baerietta* was erected for the reception of *Baerietta baeri* Hsü, 1935. At the same time, Hsü placed *Nematotaenia jägerskiöldi* Janicki, 1928 in the same genus because both forms possess similar arrangement of paruterine organs and the same number of testes (2). *Baerietta japonica* Yamaguti, 1938 also belongs in this genus.

Baerietta jägerskiöldi (Janicki, 1928) Hsü, 1935, which occurs in *Bufo regularis* Reuss. and *Rhampholeon brevicaudatus* Matschie (see Baer, 1933), is small, never reaching a length greater than 10 mm. The diameter of the strobila varies only slightly, averaging 510 microns. The mature proglottid measures only a little less than half the diameter of the strobila. The morphology of the mature proglottid is very similar to that of *N. dispar*. However, *B. jägerskiöldi* differs from *N. dispar* in the disposition of the uterus and paruterine organs. In *B. jägerskiöldi*, the uterus is extended dorso-ventrally, having relatively narrow lateral dimensions; the uterus of *N. dispar*, however, is much narrower in its dorso-ventral dimensions than in its lateral dimensions. Also there are 2 paruterine organs in *B. jägerskiöldi*, each of which contains about 20–25 eggs, while *N. dispar* contains 13–30 paruterine capsules in its gravid proglottids, each of which contains about 3 eggs.

The morphology of *B. baeri*, as described from *Bufo bufo asiaticus* Steind. in Nanking, China, closely approximates that of *B. jägerskiöldi*. However, several specific differences in morphology were noted; among these are the following: (1) a longer cirrus sac, (2) a broader scolex, and (3) broader suckers, all in *B. baeri*. Since the 2 worms have about the same diameter (50 microns), these size differences were thought to be significant. The greater length of *B. baeri* (about 23 mm as opposed to an average length of 7–9 mm in *B. jägerskiöldi*) may be partly attrib-

utable to incompletely developed material used in the description of *B. Jägerskiöldi*. The eggs in the oldest proglottids had not migrated from the basal into the apical portions of the paruterine organs—Hsü considered the termination of this migration as indicative of full development. Baer (1933) pointed out that, in *B. jägerskiöldi*, the vas efferens is connected with only 1 "testis," while this lobe led to the remaining testicular lobe via a narrow canal. This condition was not reported in *B. baeri*, there being 2 testes, each with a vas efferens.

Yamaguti's (1938) description for *B. japonica* from *Rana japonica* Günth. characterized a cestode differing from *B. baeri* in the size of the egg and from both *B. jägerskiöldi* and *B. baeri* in the possession of a spinous cuticle. Except for this spinous cuticle and the possession of paired vasa efferentia (perhaps also a slightly greater length—being as great as 27 mm in *B. japonica*), the type described by Yamaguti is very similar to *B. jägerskiöldi*.

Nematotaenia janickii Hilmy, 1936 also possesses the characteristics which are peculiar to the genus Baerietta and therefore is considered here as a member of the genus.

Baerietta janickii (Hilmy, 1936) comb. nov., from *Rappia concolor* (Hall) and *Rappia fulvovittata* (Cope) is so similar to *B. baeri* that it is very difficult to differentiate them specifically. However, the following slight differences may be noted: (1) the ventral excretory vessels in *B. baeri* measure 29 microns in diameter while they measure only 17 microns in diameter in *B. janickii*, (2) the subcuticula in *B. janickii* is quite distinctive, being a thin layer (3.8 microns), composed of triangular shaped cells whose bases abut the cuticle, (3) the testes of *B. janickii* are oval, measuring 63 microns in breadth and 46 microns in a dorso-ventral direction, while the testes of *B. baeri* are spherical, measuring 39 microns in diameter, and (4) the cirrus sac of *B. baeri* is about 90 microns long while the length of the cirrus sac in *B. janickii* is about the same as in *B. jägerskiöldi* (40 microns). Also, *B. janickii* differs from *B. jägerskiöldi* in possessing fewer eggs in each paruterine organ (only 3–5) while there are 20–25 eggs in each paruterine organ in *B. jägerskiöldi*.

In the present study another species of *Baerietta* is described. The diagnosis was based on 9 whole mounts and over 100 sets of sections.

Baerietta diana (Helfer, 1948)
(Plate I, Figs. a–g)
(All measurements in microns unless otherwise indicated)

Synonym: Proteocephalus diana Helfer, 1948.

Diagnosis: Total length of fully developed worm 5–20 mm, average 10 mm; scolex measuring 170–360 in diameter, not well defined, often merging imperceptibly with strobila; suckers subcircular to oval, 79–90 (averaging 84) in diameter; proglottids externally indistinct except at posterior end, mature segments broader than long, ranging from 272×23 to 404×37 and averaging 337×32; genital pores alternating irregularly; 2 testes in each mature proglottid, round to ovoid in cross section, averaging 59×49; ovary, though variable, essentially spherical, ventrally located, measuring from 54 to 61 (average 57) in diameter; vitelline gland saccate-spherical, measuring from 36 to 43, and averaging 39 in diameter; uterus slightly horseshoe-shaped to ovoid, averaging about 75×105, being more extensive laterally than dorso-ventrally, formed by vitelline duct; paruterine organ formation beginning about 3.40 mm posterior to the scolex, developing into 2 dorso-ventrally paired apical bulbs, each containing from 3 to 7 (average 5) eggs; cuticle possessing a layer of hair-like extensions which varies from 1.6 to 5.6 thick, with diameter of individual hairs being less than 0.4; in many specimens calcareous bodies evident adhering to cuticle.

Host: Batrachoseps attenuatus attenuatus (Eschscholtz).

Locality: Berkeley, California.
Habitat: Small intestine.
Type: U. S. National Museum.

Discussion: According to Helfer (1948) some of this worm's most distinguishing morphological features were as follows: (1) lateral bands of numerous vitelline follicles, (2) a bilobed ovary, (3) testes located in broad dorsal fields, (4) 2 bulb-like pouches of the uterine sac, and (5) paired vitelline ducts which were visible in the most posterior proglottids. The present observations concerning these points do not coincide with those of Helfer, but were correspondingly as follows: (1) single saccate ventro-posterior vitelline glands, (2) a spherical (or nearly spherical) ovary, (3) paired dorsal testes, (4) paired paruterine organs dorsoventrally disposed, and (5) 1 vitelline duct which is not visible in the most advanced posterior proglottids. The presence of posterior saccate vitelline glands simultaneously identifies the form in question as a Cyclophyllidean and not a Tetraphyllidean (or Proteocephalid). Collectively, the remaining 4 morphological characters (especially numbers 3 and 4) as indicated above, are peculiar to the cyclophyllidean family Nematotaeniidae; and the existence of these traits in the worm eliminates any possibility of placing it in the family Proteocephalidae.

There remains the possibility that *Batrachoseps attenuatus attenuatus* harbors more than 1 species of cestode. Although Helfer's types (they are in his possession) have not been examined, Dr. Marietta Voge has been kind enough to give me some stained whole mounts which Helfer had identified as *Proteocephalus diana*. These specimens are identical with those I have obtained from the same host.

SUMMARY

1. Members of the family Nematotaeniidae have previously been referred to the following as separate genera or as subfamilies: (1) Taeniidae, (2) Dilepididae, and (3) Hymenolepididae. Arguments are presented here which favor leaving nematotaeniids with full family status.

2. All of the known species of nematotaeniids are reviewed, and 2 species, one of them new, are described.

3. *Nematotaenia janickii* Hilmy, 1936 is referred to the genus *Baerietta*.

4. Four genera and 12 species are recognized in the family. These are: *Nematotaenia dispar* (Goeze, 1782); *N. tarentolae* Lopez-Neyra, 1944; *N. lopez-neyrae* Soler, 1945; *Cylindrotaenia americana* Jewell, 1916; *C. quadrijugosa* Lawler, 1938; *Distoichometra bufonis* Dickey, 1921; *D. kozloffi* nov. sp.; *Baerietta baeri* Hsü, 1935, *B. japonica* Yamaguti, 1938; *B. jägerskiöldi* (Janicki, 1928), *B. janickii* (Hilmy, 1936); and *B. diana* (Helfer, 1948).

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EXPLANATION OF PLATES

PLATE I

Baerietta diana (Helfer, 1948)

a. Scolex.
b. Mature proglottid.
c–e. Diagrams showing uterine development.
f. Proglottid with a well developed uterus.
g. The terminal proglottid.

(Figures c through g at the same magnification)

PLATE II

Distoichometra kozloffi nov. sp.

a. Mature proglottid.
b. Proglottid with a developing uterus.
c. Proglottid with developing paruterine bulbs.
d. The terminal proglottid.

(All figures at the same magnification)

C, cirrus sac; P, paruterine organ; T, testis; U, uterus; V, vitelline gland; O, ovary.

PLATE I

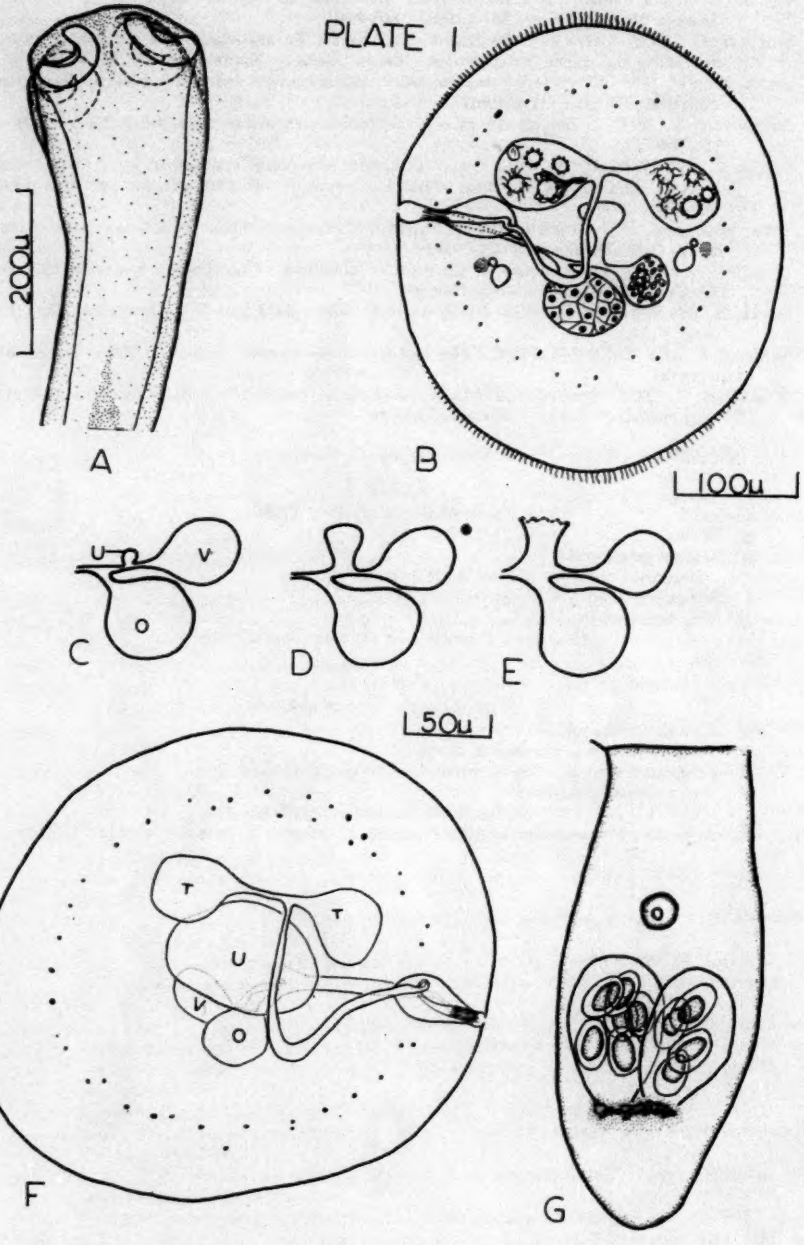
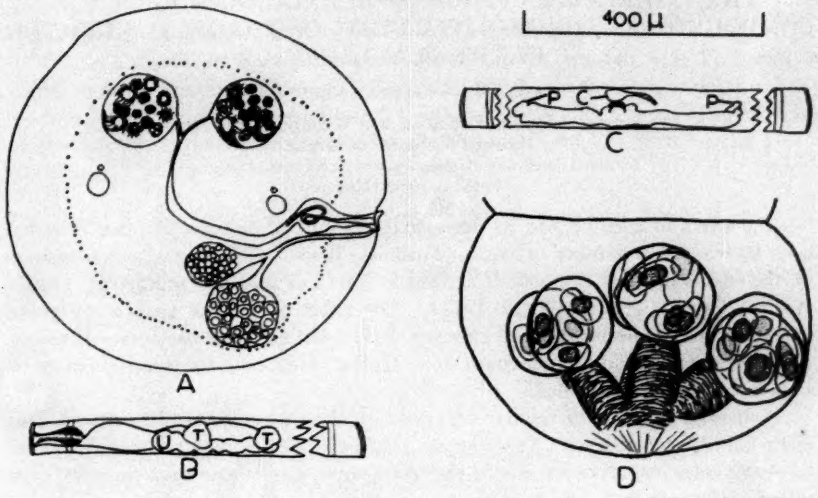


PLATE II



RESEARCH NOTE

FAILURE TO DEMONSTRATE CEREBRAL LESIONS IN RABBITS INFECTED WITH *TRYPANOSOMA GAMBIENSE*

After treating mice infected with *Trypanosoma gambiense* with subcurative doses of the amino nucleoside of puromycin following each of a series of relapses, Tobie and Highman (1956, Am. J. Trop. Med. & Hyg. 5: 504–515) reported changes in the central nervous system of the mouse comparable to those produced by trypanosomiasis in man. In the same publication they reported no unequivocally significant changes in the brain substance of 4 untreated infected rabbits and 2 infected ones which received 1 regimen of treatment, each with the same drug.

It was hoped that if rabbits were subjected to a series of subcurative treatments following succeeding relapses, a chronic infection would result, the animals would survive longer, and cerebral lesions would be produced. Chronic infections did result, the animals lived considerably longer, but no significant pathologic lesions were found in the central nervous system that could be attributed to infection with *T. gambiense* in any of these rabbits. Tissues from 15 rabbits were examined; 6 which received no treatment survived from 43 to 63 days; 1 which received treatment of the primary infection survived 95 days; 2 which were treated twice survived 87 and 95 days; 3 which received 3 regimens of treatment survived 108, 131, and 148 days; 2 which were treated 4 times survived 158 and 219 days, and finally one which developed symptoms 6 times and received treatment 6 times survived 239 days.

Following each treatment typical skin and mucous membrane lesions, pictured by Tobie and Highman (1956), regressed or disappeared, but in due time reappeared or became considerably worse as each relapse occurred and infection again became manifest. At necropsy, trypanosomes were demonstrated in the blood of 9 of the animals either microscopically in fresh drops of blood or by animal inoculation. In 2 others, which were not treated, trypanosomes had been found earlier. In 4 we failed to demonstrate parasites but symptoms were typical.—ELEANOR JOHNSON TOBIE AND BENJAMIN HIGHMAN (*with the technical assistance of* HARVEY D. JAMES, JR.), *National Institutes of Health, Bethesda, Maryland.*

THE INHIBITORY EFFECT OF ADENINE AND RELATED COMPOUNDS ON THE PROLIFERATION OF *TOXOPLASMA GONDII* IN TISSUE CULTURE*

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The effect of adenine and its derivatives on mitotic division in plant cells has been known for a number of years (Huskins, 1948). There is also information on the effects of these compounds against at least one parasitic protozoon, *Trichomonas vaginalis,* (Sprince *et al,* 1953). The effect of adenine against the poliomyelitis virus has been described (Brown, 1952), but as far as the author is aware, the effects of this and related compounds against other obligate intracellular parasites have not been recorded.

A marked delay in the cellular degeneration of tissue cultures of monkey kidney epithelial cells infected with *Toxoplasma gondii* and treated with adenine was noted in preliminary experiments where this compound was tested as a possible pyrimethamine antagonist. Further tests were made to study this phenomenon.

MATERIALS AND METHODS

Tissue Culture: Trypsinized monkey kidney tissues grown in roller tubes or on flying coverslips in roller tubes were used for all the experiments. The initial medium consisted of Hank's balanced salt solution at pH 6.8 containing 0.5% lactalbumin hydrolysate (Nutritional Biochemical Corp.), 2% calf serum (Microbiological Associates, Inc.) inactivated at 56° C for 35 minutes, with 100 units of penicillin, and 100 micrograms streptomycin per ml. After 4 or 5 days of initial growth at 37° C, the medium was changed to Earle's balanced salt solution at pH 7.6, containing 0.5% lactalbumin hydrolysate, 1% inactivated calf serum, with the same concentrations of penicillin and streptomycin (Melnick, 1955). A continuous monolayer of cells was usually formed at 4 to 7 days.

Protozoa: The RH strain of *Toxoplasma gondii* originally isolated by Doctor Albert B. Sabin from a fatal case of juvenile toxoplasmosis was used for all the experimental work. The parasite was maintained by biweekly intraperitoneal passages in mice. Suspensions of *Toxoplasma* were prepared by harvesting mouse peritoneal exudates from 14- to 20-gram NIH general purpose albino female mice infected 3 days previously with RH toxoplasmas into a 10% calf serum-Hank's solution. Organism counts were made in an AO Spenser Bright-Line haemacytometer and adjustment of the number of organisms present was made by appropriate dilution techniques using 10% calf serum-Hank's solution as diluent. These inocula were always used within a one-hour period after they were prepared.

Adenine and its Derivatives: All chemicals used were of the highest grade obtainable. Stock solutions of adenine sulfate were prepared by dissolving 25 mg adenine sulfate in 2.0 ml N/20 NaOH and bringing to 10 ml with maintenance medium. The other chemicals were freely soluble in maitenance medium. Sterilization was by Seitz filtration.

Drug Regimen: Various concentrations of adenine or adenine sulfate were placed in tissue cultures infected 4 hours previously with toxoplasmas; the chemical was allowed to remain in the tissue culture system for 5 days. At the end of the fifth day the cultures were washed 3 times, renewed with maintenance medium, reincubated, and examined daily for cellular degeneration due to the proliferation of *Toxoplasma.* Other experimental methods are discussed under their appropriate subsections. In every case at least 20 tubes of each culture group were used.

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RESULTS

The effect of adenine on the proliferation of Toxoplasma in vitro

Table I shows the lysis patterns of tissue cultures infected with *T. gondii* to which various concentrations of adenine or adenine sulfate had been added for 5-day periods. The parasite inoculum was 0.1 ml of a 1:100 dilution of peritoneal exudate removed from a mouse infected intraperitoneally with RH toxoplasmas 3 days previously.

TABLE I. *The effect of adenine and adenine sulfate on the proliferation of* T. gondii *in tissue cultures of monkey kidney epithelial cells.*

Time (days*) required to produce complete cellular degeneration in tissue cultures treated with adenine and adenine sulfate.**

Conc. (mg%)	Adenine	Adenine sulfate
0.0	5.0	5.0
0.5	5.5	8.0
1.0	11.0	8.8
2.0	11.0	10.0
4.0	11.0	12.0
8.0	11.0	15.0

* The number of days given represents the time since infection of the culture.
** The cultures were treated from day 0 (4 hours after infection) through day 5 of infection. Various lots of adenine from different sources gave similar results.

Although variations in the delay of cellular degeneration occurred, adenine in a concentration of 1.0 mg% produced approximately a 2-fold increase in the time required for lysis in the infected tissue cultures. The delay in cellular degeneration in cultures treated with similar concentrations of adenine sulfate was similar; however, this delay increased to 3-fold at 8.0 mg% dosages, while increases in the concentration of adenine alone did not have this effect. pH changes in the tissue culture tubes treated with adenine were similar to those occurring in non-treated tubes.

Additional tests on the effective range of adenine from 0.01 mg% to 16 mg% did not show a linear increase in delay in cellular degeneration within any range of drug concentrations. Concentrations below 1 mg% produced a 1-day delay; at 1 mg% to 16 mg%, there was a 5-day increase in lysis time.

Microscopic studies of the effect of adenine on the tissue culture cells and on toxoplasmas growing in the tissue cultures were also made. In these experiments, adenine was employed in a 2.0 mg% concentration in the maintenance medium and was added to cultures infected 4 hours previously with *T. gondii*. Cultures were fixed and stained with hematoxylin-eosin 12 hours after the chemical was added and at daily intervals for 6 days. Table II shows the effect of adenine on

TABLE II. *The effect of adenine on cell division and on parasite counts in tissue cultures of monkey kidney epithelium.*

Time interval after addition of adenine	No. of nuclei in various stages of division/no. of resting nuclei in tissue cultures maintained in . . .		No. of foci *Toxoplasma*/no. of host cell nuclei in tissue cultures maintained in . . .	
	2.0 mg% adenine	Maintenance medium	2.0 mg% adenine	Maintenance medium
12 hours	0/100	9/91	12/88	9/91
1 day	1/99	21/79	13/87	48/52
2 days	0/100	17/83	11/89	TNTC* (4 + lysis)
3 days	0/100	12/88	TNTC (1 + lysis)	
4 days	0/100	9/91	TNTC (2 + lysis)	
5 days	0/100	12/88	TNTC (4 + lysis)	
6 days	0/100	10/90		

* Inoculum = 2 million toxoplasmas.
TNTC = too numerous to count.

host cell division and on the parasite counts. The figures given in the tables represent the average counts made on at least 4 slides.

Monkey kidney cells undergoing mitotic division were practically absent in tissue cultures containing 2.0 mg% adenine, while in control slides of non-treated cells mitotic divisions were readily demonstrated. The number of foci of parasites increased more slowly in the adenine-treated cultures and lysis of the host cell was delayed. In control cultures of non-treated cells the foci of infection rapidly increased and cellular degeneration occurred in 2 days. The lysis time in these experiments was more rapid than in earlier experiments due to the use of a larger inoculum of toxoplasmas (2 million parasites/culture).

The effect of purine antagonists on adenine activity

Wooley (1944) reported that adenine and guanine counteracted the effects of benzimidazole. Hitchings *et al* (1948) noted that the inhibitory effect of 2,6-diaminopurine on *Lactobacillus casei* and reported that adenine completely reversed its action. It was of interest to determine if the adenine activity described above persisted in the presence of 2,6-diaminopurine and benzimidazole.

A stock solution of 2,6-diaminopurine sulfate (Nutritional Biochemical Corporation) was prepared by grinding the chemical in a mortar with maintenance medium as a diluent and allowing it to stand for 2 days at 35° C. A stock solution of benzimidazole (Merck #9R6876) was prepared in a similar fashion. Table III pre-

TABLE III. *The effect of benzimidazole and 2,6-diaminopurine on the lysis patterns of tissue cultures of monkey kidney epithelial cells infected with* T. gondii* *and treated with 2.0 mg percent adenine.*

Conc. of antagonist	Lysis time (days) of infected tissue cultures treated with . . .				
	Benzimidazole + 2.0 mg% adenine	Benzimidazole	2,6-diaminopurine + 2.0 mg% adenine	2,6-diaminopurine	Adenine
0.0	7.0	3.0	7.0	3.0	3.0
0.5	7.8	3.0	6.5	7.4	—
1.0	7.0	3.0	9.8	9.6	7.0
2.0	6.2	5.0	10.0	>14.0	—
4.0	7.4	5.0	10.0	Toxic**	—
8.0	7.0	5.3	>12.0	Toxic	—

* Cultures received an inoculum of 2 million RH toxoplasmas.
** Tissue cultures receiving 4.0 mg% or greater concentrations of 2,6-diaminopurine showed toxicity changes after 24 hours incubation.

sents the results of experiments in which these purines were supplied in the maintenance medium alone and with adenine.

The data obtained from these experiments indicated that adenine activity was not reversed by the antagonists benzimidazole or 2,6-diaminopurine, and that these antagonists themselves possessed activity similar to adenine.

The effect of delayed treatment with adenine on toxoplasmas in tissue culture

Experiments were performed to determine if a delay in cytopathogenic effect of toxoplasmas growing in tissue cultures of monkey kidney cells could be produced if adenine was administered late in the course of the infection. Tissue cultures were infected with 200,000 toxoplasmas and were incubated at 37° C in roller drums. Table IV shows the lysis times of monkey kidney cells when adenine was added to these cultures on days 1 through 5 of infection.

These data indicate that adenine produces a delay in cytopathic effect in tissue

TABLE IV. *Lysis times of tissue cultures infected with* T. gondii* *when adenine was administered late in the infection.*

Conc. of adenine (mg%)	Lysis times (days) of tissue cultures when adenine was administered on the following days after infection				
	1	2	3	4	5
8.0	13.0	13.0	12.0	12.0	6.0
4.0	13.0	13.0	12.0	12.0	6.0
2.0	13.0	13.0	10.0	10.0	6.0
0.0	6.0	6.0	6.0	6.0	6.0

* Inoculum was 200,000 toxoplasmas per tube.

cultures infected with *Toxoplasma* even when administered late in the course of the infection, but prior to the first microscopic evidence in cellular degeneration.

The effect of adenine on non-dividing toxoplasmas

Other experiments were performed to determine the effect of adenine on non-dividing toxoplasmas. These were done in a manner similar to that employed by Taylor *et al* (1952) to test a number of drugs for antimalarial activity.

It had been demonstrated previously that *Toxoplasma* will remain viable for varying lengths of time in tissue culture maintenance medium (Cook & Jacobs, 1958). If adenine were able to act against non-proliferating toxoplasmas, a difference should be detectable in the survival times of mice inoculated with parasites after exposure to adenine as compared to no exposure to this compound.

Tubes of 8.0 mg% adenine in maintenance medium and maintenance medium alone were infected with toxoplasmas so that each 0.25 ml of fluid contained 1,000 toxoplasmas. The tubes were stored at 4° C and aliquots were removed at various intervals and 0.25-ml amounts were inoculated intraperitoneally into each of 10 mice. All the mice died of toxoplasmosis, and there was no difference in their survival times. Hence it can be concluded that the effect of adenine in tissue culture is not due to direct toxicity and is not exerted against non-proliferating parasites.

Penetration of Toxoplasmas into adenine-treated cells

To determine if adenine interfered with the penetration of monkey kidney cells by the parasite, microscopic studies were performed using coverslip cultures grown in 8.0 mg% adenine in maintenance medium and in maintenance medium only. An average of 12 toxoplasmas was found per 100 host cell nuclei in cultures which were treated with adenine, infected with toxoplasmas after 1 day, and examined 4 hours after infection. In control cultures grown in maintenance medium only, an average of 9 parasites was found per 100 host cell nuclei after 4 hours' incubation. These studies indicate that there is no major difference in the numbers of parasites that could enter cells treated with 8.0 mg% adenine and in those kept with maintenance medium alone.

The failure of monkey kidney cells to "store" adenine

Monkey kidney cells were treated with 8.0 mg% adenine for 1 week, washed 3 times with Earle's solution, and renewed with maintenance medium. These cultures then received a challenge with 200,000 toxoplasmas. Both adenine-treated and control cells lysed in 6 days indicating that adenine activity was apparent only when the chemical was present in the medium. Thus, storage of the chemical in an active form did not take place and the effect of the chemical was only temporary.

The effect of adenine derivatives on the proliferation of toxoplasmas

Additional experiments were performed using the nucleoside, adenosine, the nucleotides, adenylic acid (AMP) and adenosine triphosphate (ATP), and the oxypurine, xanthine. Table V summarizes the results of these experiments.

TABLE V. *The effect of a nucleoside and nucleotides of adenine and xanthine on the proliferation of* T. gondii* *in tissue cultures of monkey kidney epithelium.*

Conc. (mg%)	Lysis time (days) of infected tissue cultures treated with...			
	Adenosine	Adenylic-5-phosphoric acid (AMP)	Adenosine-triphosphate	Xanthine
0.0	3.2	3.0	3.0	5.0
0.5	4.8	4.8	4.8	5.0
1.0	5.0	5.2	6.0	5.0
2.0	5.0	7.0	6.0	5.0
4.0	5.0	6.6	13.0	7.6
8.0	5.2	6.8	9.2	7.0

* The tubes containing adenosine, AMP, and ATP received inocula of 2 million RH toxoplasmas. The tubes containing xanthine received an inoculum of 200,000 RH toxoplasmas.

The data indicate that adenosine, AMP, ATP, and xanthine all act to delay the cellular degeneration of tissue cultures infected with *T. gondii.*

DISCUSSION

Toxoplasma failed to divide at normal rates in the presence of the compounds tested in the experiments described above. This is shown by the counts made of the numbers of parasites present in both treated and non-treated cells.

The microscopic studies indicated that the purine activity was not due to changes in the permeability of host-cell membranes brought about by the addition of adenine and subsequent inability of all of the parasites to enter the cells. It is apparent, from the experiment testing the effect of adenine on non-proliferating toxoplasmas, that the purine had no direct toxic effect. The decrease in the numbers of host cell nuclei showing mitotic division after treatment with adenine suggests that the delay in cytopathic effect in these cultures may be associated with modifications in the metabolism of the host cell and is possibly secondary to them. Renate Lettré (1954) reported that vacuolization of the cytoplasm of mesenchymal tissue of chick embryos in tissue culture can be brought about either by the addition to the medium of ammonium salts or salts of organic amines, or of any substance yielding free ammonia during intracellular degradation, and stated that with the addition of adenine (1 mg/ml) the cell surface became less "tight" than normal and some cells showed a bubbling of the cell surface without going into mitosis. Hans Lettré (1954) noted a rigidity of fibroblastic cells on the addition of 10^{-2}M ATP and reported that this was reversible as the ATP was degraded in the medium. In regard to the present study, if an active product is formed from adenine or the other purines, it must be depleted rapidly as can be seen from the experiments in which cells treated with adenine and then washed free of the chemical were lysed as rapidly as controls.

Benzimidazole and 2,6-diaminopurine rather than reversing the effect of adenine gave effects similar to it. The possibility exists, therefore, that the action of these compounds is due to some general "poisoning" effect, either directly or thru breakdown products, rather than to interference with some specific metabolic activities of the cells or parasites.

SUMMARY

1. Adenine, adenosine, adenylic-5-phosphoric acid, adenosine-triphosphate and xanthine possess the ability to delay the cytopathogenic effect of *T. gondii* in tissue culture.

2. Adenine or derivatives of adenine are not stored within the host cells when concentrations of 2.0 mg% or less are used and the effect of this purine is demonstrable only when it is present in the medium.

3. The effect of these compounds could be demonstrated only against proliferating toxoplasmas.

4. The ability of adenine to cause a delay in inhibition of mitotic division of the parasites and the possibility of the conversion of the adenine into an actively toxic product (ammonia) which is rapidly degraded within the host cell have been discussed. Furthermore, this study shows that the effects of these compounds on *Toxoplasma* are associated with a depression of mitotic activity of the host cell.

5. It was noted that adenine in 2.0 mg% to 16.0 mg% concentrations caused a decrease in the numbers of host cell nuclei showing mitotic division.

6. The adenine activity was not reversed by the purine antagonists, benzimidazole or 2,6-diaminopurine, and these antagonists themselves also were shown to have activity similar to adenine.

7. Adenine apparently does not interfere with the penetration of toxoplasmas into host cells.

8. Adenine produced a delay in cytopathic effect in tissue cultures infected with a light *Toxoplasma* inoculum even when administered as late as the fourth day of infection.

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IN VITRO INVESTIGATIONS ON THE ACTION OF PYRIMETHAMINE AGAINST *TOXOPLASMA GONDII*

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The marked activity of 2,4-diamino-5-*p*-chlorophenyl-6-ethyl-pyrimidine or pyrimethamine (Daraprim) against toxoplasmosis in mice has been reported by Eyles and Coleman (1952), independently by Summers (1953), and has been confirmed by Trevino, Varela and Palencia (1953), and by Beverley and Fry (1957). The purpose of the present report is to describe the minimal effective concentration of pyrimethamine that causes inhibition and death of proliferating toxoplasmas growing in tissue cultures of monkey kidney cells and embryonic mouse mince and to present certain experimental data relative to the mode of action of pyrimethamine against *Toxoplasma gondii* in a tissue culture system.

MATERIALS AND METHODS

Mouse mince tissue cultures were prepared by the method of Chernin and Weller (1954). Monolayer tissue cultures of monkey kidney cells were prepared and maintained by the method of Melnick (1955).

The methods of handling the parasite and the criteria for evaluating the cytopathic effect of *Toxoplasma* in tissue culture have been described elsewhere (Cook and Jacobs, 1958). The RH strain of *T. gondii* was used throughout.

Daraprim, a brand of pyrimethamine, was supplied through the courtesy of Dr. G. H. Hitchings of The Wellcome Research Laboratories, Burroughs Wellcome and Company (U.S.A.), Inc., Tuckahoe, New York. Stock solutions of 100 mg% pyrimethamine were prepared by bringing 100 mg of pyrimethamine dissolved in 2.0 ml of lactic acid, U.S.P. (85%) to 100 ml with maintenance medium. The drug solution was sterilized by Selas filtration and was stored at 4° C. All other solutions of pyrimethamine were prepared from such stock solutions by dilution methods.

Sulfadiazine solutions were prepared in maintenance medium from vials of sterile sodium sulfadiazine (Lederle), 0.25 gm/ml.

RESULTS AND DISCUSSION

Determination of the Minimal Effective Concentrations of Pyrimethamine That Cause Inhibition of Proliferating Toxoplasmas

The first experiments were performed on the minimal toxoplasmicidal concentration of pyrimethamine in mouse mince tissue cultures. Eight-day cultures of mouse mince were infected with toxoplasmas and groups of the cultures were treated ½ hour later with graded concentrations of pyrimethamine in maintenance medium. After 7 days incubation, inoculations of the supernatant medium and suspensions of the explants were made into mice to determine the presence of viable organisms. Survival days of the mice were recorded. At the same time other groups of the cultures were washed 3 times with maintenance medium and were reincubated in drug-

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free medium and examined for cellular degeneration due to proliferation of the toxoplasmas. In order to rule out any possible activity of the lactic acid in which the drug was dissolved, control cultures were prepared using similar amounts of lactic acid and were given identical inocula of toxoplasmas.

Table I gives a summary of the results of 2 typical experiments. At a concentration of 0.1 mg%, pyrimethamine inhibited proliferation of *Toxoplasma*, as indicated by the delay in lysis time of the tissue cultures and in death of mice inoculated with culture materials. The effect of the drug at this concentration was not permanent; removal allowed proliferation of toxoplasmas to continue and complete cellular degeneration of tissue cultures occurred by day 14, 7 days after replacement of the medium. The concentration of 0.3 mg%–0.4 mg% pyrimethamine approximated the minimal effective dose for complete clearance of parasites from mouse mince cultures. Lactic acid, used alone at concentrations necessary to dissolve pyrimethamine, had no effect on the parasites or the host cells. Examination of cultures receiving only drug solutions showed no visible damage to the cells when these concentrations of pyrimethamine were used.

TABLE I. *The toxoplasmicidal effect of pyrimethamine in mouse embryo mince tissue cultures using an exposure time of 7 days*

Concentration of Pyrimethamine (mg%)	Number of toxoplasmas inoculated	Number of mice dead of 5 inoculated with . . .		Cytopathic effect in tissue cultures on . . .	
		Supernatant	Explants	Day 7	Day 14
1.00	2×10^6	0	0	0	0
0.40	"	0	0	0	0
0.30	"	2 (8, 8)*	2 (8, 8)	0	0
0.10	"	1 (7)	5 (7, 7, 7, 7, 8)	1+	4+
0.01	"	5 (8, 8, 8, 8, 8)	5 (8, 8, 8, 8, 8)	4+	ND**
0.00	"	5 (8, 8, 8, 8, 8)	5 (8, 8, 8, 8, 8)	4+	ND
0.00 (Lactic Acid Control)	"	5 (8, 8, 8, 8, 8)	5 (8, 8, 8, 8, 8)	4+	ND

* Figures in parenthesis indicate the survival days of the mice.
** Test not performed.

Later experiments were performed to determine the minimum effective dose of pyrimethamine against *T. gondii* in tissue cultures of monkey kidney epithelial cells. In these experiments a standard inoculum of 2,000,000 toxoplasmas was used throughout, and the drug was added to the cultures approximately 4 hours after infection. The criteria for these tests were microscopic observations of the tissue cultures for cytopathic effects and the results of inoculations into mice of the cell suspensions from the cultures. These experiments were designed to show the effect of pyrimethamine especially in relation to the time of drug exposure required to destroy the parasite with different concentrations of the drug. The results of 1 of 6 such experiments are shown in Table II.

As little as 0.005 mg% pyrimethamine for 12 hours caused a delay in lysis of monkey kidney cultures infected with *Toxoplasma*. It was noted, however, that although 0.025 mg% pyrimethamine for 3 days effected a complete "kill" of the toxoplasmas in some instances, an occasional tube with a higher concentration of pyrimethamine or a longer exposure time became positive for *Toxoplasma*. This may be an indication of the occasional presence of latent parasites, of the relative ease of development of pyrimethamine-resistant strains of *Toxoplasma*, or may be simply a demonstration of the "selecting out" of pyrimethamine-resistant strains of the organism. Alternate passage of these toxoplasmas through mice and then in tissue culture failed, however, to result in the isolation of a resistant strain.

TABLE II. *The toxoplasmicidal effect of pyrimethamine in monkey kidney epithelial tissue cultures*

Concentration of pyrimethamine (mg%)	Drug exposure time (Days)	No. of tubes lysed of 5 inoculated	Maximum delay in lysis time (Days)	Results of mouse inoculations
0.000	0.0	5	0	+
0.001	0.5	5	0	+
	1.0	5	0	+
	2.0	5	0	+
	3.0	5	0	+
	4.0	5	0	+
0.005	0.5	5	1	+
	1.0	5	2	+
	2.0	5	2	+
	3.0	5	3	+
	4.0	5	3	+
0.010	0.5	5	1	+
	1.0	5	2	+
	2.0	5	2	+
	3.0	5	3	+
	4.0	5	3	+
0.025	0.5	5	2	+
	1.0	5	5	+
	2.0	5	5	+
	3.0	0	–	+ (½)*
	4.0	0	–	–
0.050	0.5	5	5	+
	1.0	0	–	+
	2.0	2	2	+
	3.0	0	–	–
	4.0	0	–	–
0.100	0.5	5	–	+
	1.0	0	–	+
	2.0	0	–	+
	3.0	0	–	+ (½)
	4.0	0	–	–
0.150	0.5	0	–	+
	1.0	0	–	+
	2.0	0	–	+
	3.0	0	–	–
	4.0	0	–	–
0.250	0.5	0	–	+
	1.0	0	–	+
	2.0	0	–	+
	3.0	0	–	–
	4.0	0	–	–
0.500	0.5	0	–	+
	1.0	0	–	+
	2.0	0	–	+ (½)*
	3.0	0	–	–
	4.0	0	–	+ (½)*
1.000	0.5	0	–	+
	1.0	0	–	+
	2.0	0	–	+ (½)*
	3.0	0	–	–
	4.0	0	–	–

* One-half of the number of mice inoculated died from toxoplasmosis. The remainder survived.

It was also demonstrated that a 3-day exposure to drug was the shortest time interval found to make the cultures non-infective for mice despite the concentration of pyrimethamine used. The delay in cellular degeneration produced by sub-lethal doses of the drug suggests that pyrimethamine acts by interfering with the multiplication of the toxoplasmas. Experimental evidence to support this supposition is given in another section. It is noteworthy that cytopathic effect is not entirely reliable as an indication of the effectiveness of pyrimethamine in tissue cultures.

It is not possible to compare the preliminary data obtained with mouse mince cultures with those on the monkey kidney epithelium cultures because the former contained a greater amount of tissue which could conceivably bind drug, and because the exposure times were different. The effect of the drug was, however, certainly more pronounced in the latter cultures, as evidenced by delay in lysis times in the presence of much smaller concentrations of pyrimethamine.

TABLE III. *The effect of pyrimethamine on toxoplasmas in tissue cultures of monkey kidney epithelial cells*

Time interval after drugging (Days)	*Toxoplasma* + 0.05 mg% pyrimethamine	*Toxoplasma* Control
0.5	32/68*	14/86
1.0	17/83	49/51
2.0	3/97	TNTC,** no lysis
3.0	0/100	TNTC, 4+ lysis
4.0	0/100	TNTC, 4+ lysis

* Foci of *Toxoplasma* infection/number of host cell nuclei.
** Toxoplasmas were too numerous to count.

Microscopic studies

Smith (1952) has described morphological changes in growing ring trophozoites of *Plasmodium falciparum* after the administration of pyrimethamine. Microscopic studies of the effect of pyrimethamine on *Toxoplasma* in tissue cultures of monkey kidney epithelial cells also were made in this investigation. Coverslip cultures of monkey kidney cells were inoculated with 2,000,000 toxoplasmas and incubated for 4 hours in roller drums at 37° C. The cultures were then treated with 0.05 mg% pyrimethamine and reincubated. Four cultures were washed in maintenance medium and fixed in Bouin's solution at the end of 12 hours and 1, 2, 3, and 4 days. They were then stained with hematoxylin-eosin. Table III summarizes the counts

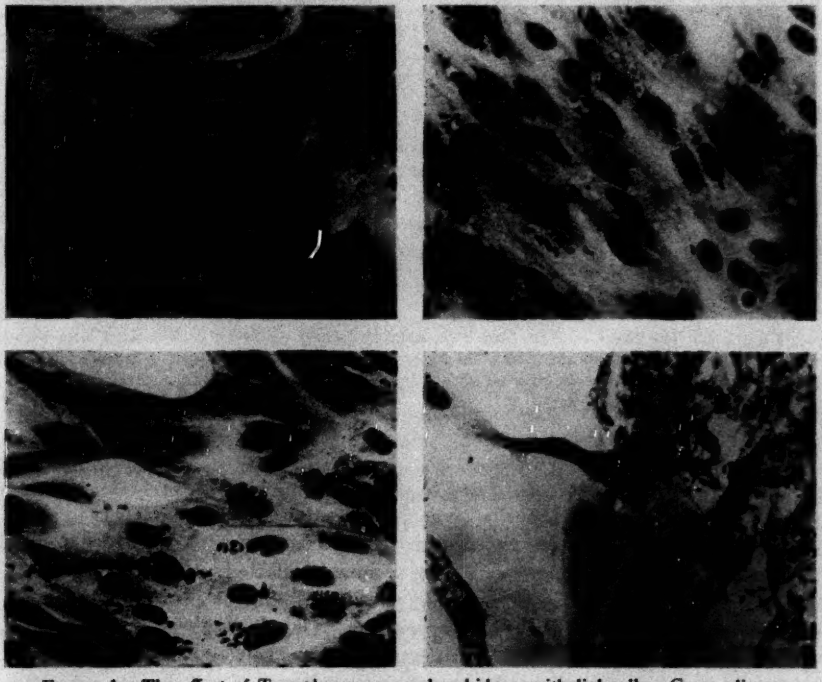

FIGURE 1. The effect of *Toxoplasma* on monkey kidney epithelial cells. Cover slip preparation. Hematoxylin-eosin, ×600. Normal monkey kidney epithelial cells. (A) Monkey kidney cells from a culture infected with 2,000,000 toxoplasmas, day 1 of infection (B), day 2 (C), day 3 (D).

of foci of *Toxoplasma* infection per number of host cell nuclei in at least 5 areas of each of these cultures.

The data clearly show the effective freeing of infected cells of the parasite. Figures 1 and 2 show treated and non-treated cultures at various time intervals. No microscopic evidence of any harmful effects of the drug on infected or uninfected cells was apparent.

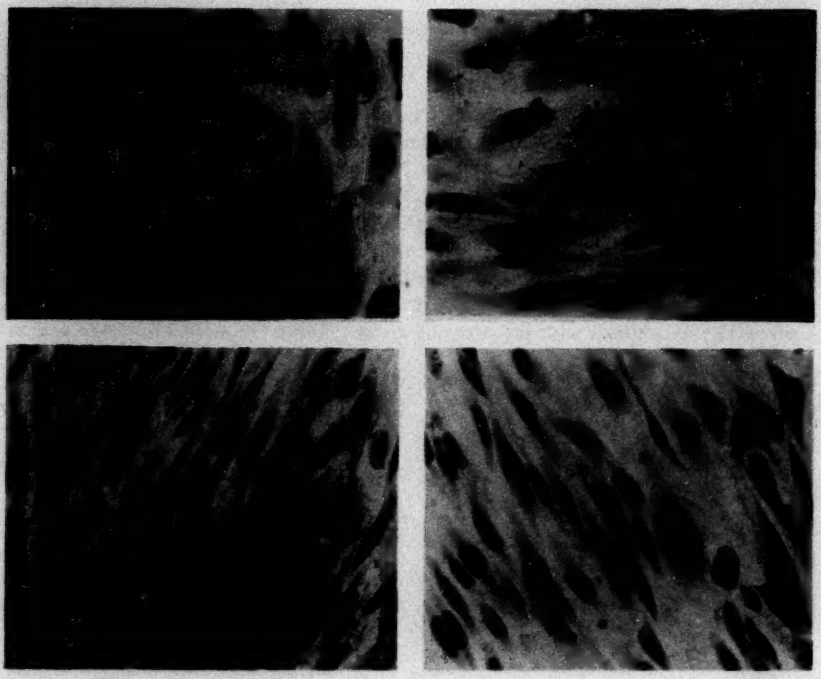

FIGURE 2. The effect of *Toxoplasma* on monkey kidney epithelial cells treated with pyrimethamine. Hematoxylin-eosin, ×600. Monkey kidney epithelial cells from a culture infected with 2,000,000 toxoplasmas and treated with 0.5 mg% pyrimethamine, 12 hours after infection (A), day 1 (B), day 2 (C), day 3 (D).

Toxicity levels of pyrimethamine

Two experiments were performed to determine the toxicity of pyrimethamine for monkey kidney cells in tissue cultures. Because the drug was dissolved in lactic acid, lactic acid controls were tested also. At concentrations of drug higher than 10 mg%, the pH of the culture fluid was noticeably affected and morphologic evidence of this change was apparent in the cells exposed to concentrations of pyrimethamine greater than 15 mg%. The monkey kidney cells exposed to 50 mg% pyrimethamine for 5 days, however, showed no greater effects of toxicity than the lactic acid controls. There was no morphologic evidence of cell damage when the drug was used in concentrations of 0.5 mg% for 1 week and pH changes in these cultures proceeded at normal rates. Furthermore, new cultures made by splitting

cultures and transplanting the cells to new tubes showed growth rates for treated cells similar to non-treated cells.

The effect of pyrimethamine on non-proliferating toxoplasmas

It has been demonstrated in other studies that *Toxoplasma* will remain viable for brief periods of time in tissue culture maintenance medium (Cook and Jacobs, 1958). If pyrimethamine were able to act against non-proliferating parasites, a difference should be detectable in the survival times of mice inoculated with parasites after exposure to pyrimethamine as compared to no exposure to the drug.

Tubes of medium containing 0.4 mg% pyrimethamine and control tubes without drug were inoculated with 100, 1,000, or 10,000 toxoplasmas per 0.25 ml of fluid. The tubes were maintained at 4° C. At 1, 3, 5, and 24 hours, groups of 10 mice were inoculated intraperitoneally with 0.25 ml of the treated and non-treated suspensions and survival times of the mice in each group were noted. The results of one of these experiments are presented in Table IV.

TABLE IV. *The effect of pyrimethamine on non-proliferating toxoplasmas*

No. of Toxos	No. of mice dead of 10 inoc. from tubes with 0.4 mg% pyrimethamine at various hours				No. of mice dead of 10 inoc. from tubes with maintenance medium at various hours			
	1 hr.	3 hrs.	5 hrs.	24 hrs.	1 hr.	3 hrs.	5 hrs.	24 hrs.
100	10(7.8)*	7(8.4)	9(9.0)	2(10)	10(7.7)	7(8.7)	8(9.4)	0
1,000	10(7.4)	10(7.9)	10(8.1)	9(8.2)	10(7.3)	9(7.9)	10(8.2)	8(10.1)
10,000	10(6.3)	10(6.9)	10(7.8)	10(8.9)	10(7.1)	10(7.3)	10(7.9)	8(8.0)

* Figures in parentheses are the average survival times of the mice in days.

These data indicate that pyrimethamine exerts no effect on non-proliferating toxoplasmas. This result may be explained either by the inability of the drug to act against non-dividing forms of the parasite or by the necessity of activation of the drug in some manner within the host cell before toxoplasmicidal activity can be demonstrated. It is interesting to note that in *in vitro* studies of the effects of drugs on surviving malarial parasites, Greenberg *et al* (1952) noted that pyrimethamine had no detectable activity.

The effect of pyrimethamine on penetration of Toxoplasma *into cells*

Experiments to determine relative penetration times of toxoplasmas into host cells in the presence and in the absence of the drug were performed. Two hundred thousand toxoplasmas were inoculated into roller tubes of monkey kidney epithelium in the presence of 0.5 mg% pyrimethamine and in maintenance medium only. At one-half hour intervals the cultures were washed 3 times and new medium was added to ½ of the tubes in each group for determination of lysis times. The cells from the remaining tubes were pooled and inoculated into mice to determine survival times.

The lysis time of the treated cultures was 9.0 days following exposure for 1 hour to toxoplasmas and drug; after a 1.5-hour exposure, no cytopathic effect occurred in 21 days of incubation. The untreated cultures exposed for 0.5–1.5 hours to toxoplasmas lysed in 7.5 days, and those exposed for 2 or more hours lysed in 6 days. The failure of cultures to lyse following exposure to pyrimethamine and toxoplasmas for these short intervals is probably attributable to rapid accumulation and storage of the drug within the host cell in concentrations sufficient to inhibit the parasite

(Cook and Jacobs, in press). The tissue cultures which had not lysed by day 21 were found negative for toxoplasmas on mouse inoculation. The mice inoculated from both treated and non-treated cultures died in an average of 7.0 to 7.5 days, regardless of exposure time. These data on survival time of mice inoculated from the tissue cultures suggest that toxoplasmas are equally able to penetrate cells in the presence or absence of drug.

The effect of delayed treatment with pyrimethamine

Embryonic mouse mince tissue cultures were infected with 1,000,000 toxoplasmas and pyrimethamine was added to the medium at various time intervals after infection. A 0.3 mg% concentration of pyrimethamine and a 7-day drug exposure time were used throughout. No lysis of these cultures occurred when the drug was added as late as 48 hours after infection. Eight of 10 cultures treated 4 days after infection lysed in 13 days as contrasted with 7 days for cultures treated on day 6. Table V presents the results obtained when this experiment was repeated

TABLE V. *The effect of a 48-hour delay in treatment with pyrimethamine on* T. gondii* *growing in tissue cultures of monkey kidney epithelium*

Conc. of pyrimethamine (mg%)	Cytopathic effect in tissue culture in a 25-day period	No. of mice dead of 8 inoc. from tissue culture	
		Immediately after treatment	7 days after termination of treatment
0.00	4+ lysis in 5 days	8 (7)**	ND***
0.05	0	4 (9.5)	0
0.10	0	5 (10.8)	0
0.30	0	8 (12.0)	0
0.50	0	5 (10.0)	0

* Tubes received an inoculum of 200,000 RH toxoplasmas.
** Figures in parentheses are the average survival days of the mice.
*** Not done.

in tissue cultures of monkey kidney epithelial cells. In these experiments, mouse inoculations were performed from tissue cultures immediately after the 7-day drugging period and 7 days after the termination of the drugging period.

These experiments indicated that treatment with pyrimethamine could be delayed for 48 hours after infection and still be effective in stopping proliferation of parasites. Proliferation of the protozoon was stopped even when pyrimethamine in concentrations as low as 0.05 mg% was added to the cultures 48 hours after the infection was initiated. Mouse inoculations performed from these cultures immediately after the termination of drugging were positive, however, indicating that viable parasites remained in the cultures. The mouse inoculations made from the cultures 7 days later were uniformly negative, suggesting that long continued exposure to drug stored even at low concentrations eventually kills the parasite.

The effect of the age of the tissue culture system on the effectiveness of pyrimethamine

Although it has been demonstrated (Cook and Jacobs, 1958) that the age of host cell apparently plays no role in the ability of a cell to support the growth of *Toxoplasma,* it was felt that changes in host cell metabolism due to the effects of age might change the ability of pyrimethamine to clear cultures of parasites. When a standard inoculum of 2,000,000 toxoplasmas and a drug exposure of 5 days at 0.5 mg% concentration were employed, 7, 14, and 28-day cultures of monkey kid-

ney cells were uniformly cleared of the parasite as demonstrated by examination of the tissue cultures for cytopathic effects and by the results of mouse inoculations from these cultures. These experiments indicated that the age of the host cell has no effect on the ability of the drug to clear tissue cultures of the parasite and suggest that if intracellular activation of the drug is required this activation proceeds at similar rates in young, actively proliferating cells and in old cells.

The synergistic effect of pyrimethamine and sulfadiazine against T. gondii *in vitro*

The synergistic action of pyrimethamine and sulfadiazine against murine toxoplasmosis was reported by Eyles and Coleman (1953; 1955) and has been confirmed by Beverley (personal communication to Dr. D. E. Eyles).

The effect of pyrimethamine and sulfadiazine, alone, and in combination, on the RH strain of *Toxoplasma* growing in roller tubes cultures of embryonic mouse mince was studied in 1 experiment. While cultures treated with 0.01 mg% pyrimethamine or 0.05 mg% sodium sulfadiazine lysed in 7 and 11 days respectively, only 1 of 10 cultures treated with both these drugs at the same concentrations showed lysis in 11 days and the others showed no cytopathic effect in the 13-day observation period.

GENERAL DISCUSSION

The actual definition of the amounts of pyrimethamine required to produce definitive cure has been accomplished by the tissue culture methods described above and synergism with sulfadiazine has been noted *in vitro*. These findings, together with the findings of Smith and Ihrig (1957) on blood levels of the drug in monkeys and humans in relation to time, contribute to a rationale for treatment of systemic human toxoplasmosis. They are also basic to establishing dosages in ocular toxoplasmosis, when information is obtained on the penetration of drug into the eye.

It has been noted above that whereas generally very low doses of pyrimethamine were effective in clearing tissue cultures of *Toxoplasma*, occasionally cultures exposed to much higher concentrations of the drug remained infective to mice. No evidence was obtained that the parasites from such cultures were resistant to pyrimethamine, although it has been possible to develop resistant lines of *Toxoplasma* with certain procedures (Cook, to be published). The possibility may be considered, therefore, that under some circumstances dormant forms of the parasites may persist in cultures cleared of proliferating toxoplasmas by pyrimethamine. This hypothesis suggests that it may be profitable to study *in vitro*, by the use of drugs and immune serum, the mechanism by which pseudocysts are formed.

The ability of pyrimethamine to eradicate *Toxoplasma* from tissue cultures suggests a use for this system in the study of cellular immunity *in vitro*. Actually, the work prosecuted above was performed with this purpose in mind, and a number of experiments were done with cultures cleared of *Toxoplasma* by the drug. These led to the finding of storage of pyrimethamine by cells *in vitro*, which will be reported in detail elsewhere. Since studies of antagonism to pyrimethamine (Cook, to be published) have shown the reversal of the action of this drug by certain metabolites, a technique has become available for clearing cultures of parasites and for reversing residual drug. Thus, requirements for studying cellular immunity or antibody-production *in vitro* can be satisfied by this system. Together with the use of cultures of uniformly susceptible cells, such a system may, therefore, offer us a valuable tool for immunological investigation.

SUMMARY

1. The minimal effective concentrations of pyrimethamine that cause inhibition and death of proliferating toxoplasmas growing in tissue cultures of monkey kidney cells and embryonic mouse mince have been determined. An exposure of 4 days to a concentration of 0.25 gamma per ml of the drug was sufficient not only to prevent lysis of the monkey kidney cells but to render the cultures free of the parasite.

2. The toxicity level of pyrimethamine in tissue cultures of monkey kidney was determined. No morphologic evidence of cell damage was evident when the drug was used in concentrations of 0.5 mg% for 1 week.

3. Pyrimethamine had no effect on non-proliferating toxoplasmas or on the ability of *Toxoplasma* to enter tissue culture cells.

4. Even after 1.5 hours' exposure of the host cells to 0.5 mg% pyrimethamine, conditions within the cell were not favorable for the proliferation of toxoplasmas and prolonged incubation of the cultures so treated showed eventual clearing of the infection.

5. Pyrimethamine treatment could be delayed for 48 hours after infection of tissue cultures and still be effective in clearing the cultures of the parasite.

6. The age of the host cell had no effect on the ability of the drug to clear tissue cultures of the parasites.

7. The synergistic effect of pyrimethamine and sulfadiazine against *T. gondii* has been confirmed *in vitro*.

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THE PRODUCTION OF CERCARIAE BY A STRIGEID MOTHER SPOROCYST *

KATHLEEN L. HUSSEY, WILLIAM W. CORT AND DONALD J. AMEEL

During a routine examination for larval trematodes of a collection of *Lymnaea stagnalis appressa* Say from Douglas Lake, Michigan, a snail was discovered that harbored a double infection of strigeoids, *viz, Diplostomum flexicaudum* (Cort and Brooks, 1928) and *Cotylurus flabelliformis* (Faust, 1917). A mother sporocyst of *C. flabelliformis* was found between the body and digestive gland of this snail and was removed intact (Fig. 1). It was almost 2 inches in length and contained numerous daughter sporocyst embryos and germinal masses. In addition there were in it 2 fully developed metacercariae (tetracotyles) and 7 well developed cercariae and 6 cercarial embryos. The tetracotyles had evidently developed from cercariae of *C. flabelliformis* which had penetrated from the outside, since tetracotyles of this species are commonly found in the germinal sacs of trematodes in this area. The presence of fully developed cercariae and cercarial embryos inside of this mother sporocyst (Figs. 2 and 3) can only be explained by postulating that they had developed from germinal cells produced by the mother sporocyst. We know of no other instance where daughter sporocysts and cercariae have been found in the same mother sporocyst.

Rediae producing both daughter rediae and cercariae have been reported by a number of workers for trematode species belonging to several different groups. Thomas (1883) in his description of the larval stages of *Fasciola hepatica* pictured a redia containing both rediae and cercariae. Looss (1896) found both cercariae and rediae in rediae of *Gastrodiscus aegyptiacus*. In more recent work the same thing has been found in a number of species, for example, in *Ribeiroia ondatrae* (Price, 1931) by Beaver (1939), in *Zygocotyle lunata* (Diesing, 1836) by Willey (1941), in *Heterophyes aequalis* Looss (1902) by Kuntz and Chandler (1956) and by Wootton (1957) in a species of *Allocreadium*.

Since the relations of the germinal cells in mother sporocysts and mother rediae are essentially the same, it is not surprising to find cercariae developing along with daughter sporocysts in a mother sporocyst. However, we know of no other report of production of both daughter sporocysts and cercariae by a mother sporocyst.

In this snail most of the digestive gland was filled with mature daughter sporocysts of *D. flexicaudum* which were producing large numbers of cercariae. This and the fact that the infection with *C. flabelliformis* was immature indicated that the infection with *Diplostomum* entered the snail before that of *C. flabelliformis*. In the infection with *C. flabelliformis* the mother sporocyst contained large numbers of embryos of daughter sporocysts, and the few that had escaped were still immature.

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It may be that the crowding of the digestive gland with sporocysts of *D. flexicaudum*, which had left no space for the normal development of the sporocysts of *C. flabelliformis*, stimulated the mother sporocyst to produce cercariae. Since mother sporocysts are so infrequently examined it may well be that the production by them of cercariae is not unusual and has been overlooked.

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RESEARCH NOTE

POTASSIUM HYDROXIDE—CENTRIFUGATION METHOD FOR DETECTION OF *SCHISTOSOMA MANSONI* EGGS IN FECES

One of the currently recommended techniques for detection of *Schistosoma mansoni* eggs in feces is the sodium sulfate-*Triton*-ether-centrifugation test. This is relatively complex and expensive as a mass testing procedure. Preliminary trials have indicated that a simple KOH-centrifugation method has certain advantages over the above technique.

In the method presented 5 ml of a 5% KOH solution are well mixed with approximately 1 g of the fecal sample and allowed to stand for a period of " digestion" of 1 hour at room temperature. Then the sample is filtered through gauze and centrifuged at low speed (1200 rpm). The sediment is examined microscopically for helminth ova.

The new method provides a microscopic field in which the schistosome eggs are sharply outlined and easily distinguished from extraneous matter. The method is simple, inexpensive, and speeds the reading of preparations. Paired tests with 276 fecal samples comparing both techniques were made. Results show that the KOH method has the same general efficiency as the sodium sulfate-*Triton*-ether-centrifugation technique in schistosomiasis diagnosis. Of the 276 tests performed, 123 were positive by the routine method and 106 by the KOH method, while 79 were positive by both methods.—Frederick F. Ferguson, Aida Z. de Colon, *Puerto Rico Field Station, Communicable Disease Center, U. S. Public Health Service,* and Maria V. Zayas, *Bureau of Environmental Sanitation, Commonwealth of Puerto Rico Department of Public Health, San Juan.*

PLATE I

EXPLANATION OF PLATE

FIGURE 1. Entire mother sporocyst
FIGURE 2. detail of area "a"
FIGURE 3. detail of area "b"
c—cercaria; c e—cercarial embryo; d s—daughter sporocyst; m—metacercaria.

BLOOD-INDUCED BLACKHEAD

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The blood phase of the life cycle of *Histomonas meleagridis*, the protozoan parasite which produces a commonly fatal disease of the liver and ceca of turkeys called blackhead or histomoniasis, has been postulated but incompletely proven. Durant (1929), uniquely demonstrated the ceca to be the primary site of natural blackhead infection when no infection resulted in turkeys whose ceca were surgically abligated, or removed, prior to exposure with embryonated *Heterakis gallinae* ova. Farmer and Stephenson (1949) indicated a definite time relation between the development of the infection in the ceca and the appearance of liver lesions. No positive proof of the presence of these protozoa in the blood stream has been published, but thus far no other hypothesis has been advanced for its migration from this site of infection in the ceca to the liver. Assuming that an infective form might be found in hepatic portal blood taken during this critical period, the following experiments were initiated.

MATERIALS AND METHODS

Adult turkeys showing such early symptoms of blackhead as weight retardation and the appearance of cecal cores in the droppings were chosen as suitable donor birds for these experiments. Laparotomies were performed on the anesthetized donor birds, revealing mild developing liver lesions and large ulcerated cecal lesions of blackhead. Various amounts of whole blood were withdrawn from the following sites into sterile citrated syringes: (1) 1 to 10 ml from the posterior mesenteric vein (at a point about midway along the large intestine), (2) 1 to 2 ml from any of the small cecal veins which drain the ceca and merge with the dorsal tributary of the portal vein, and (3) 4 to 5 ml from the heart or wing vein. Blood smears were made before these blood samples were inoculated into the experimental recipient poults.

Inoculated birds which subsequently exhibited symptoms were observed closely each day for abnormal cecal droppings or other signs of disease. At the time of death or sacrifice all birds were examined for lesions or complicating infections, and portions of affected liver were utilized for passing this infection cloacally into other susceptible poults by the method of Sautter, Pomeroy and Roepke (1950).

All birds were autopsied as soon as possible after death and when found suitable, slices of necrotic or suspected tissues were fixed in Bouin's or 10% formalin solutions. Dehydration, clearing and embedding in paraffin were accomplished in the usual way and sections 6 to 10 microns thick were cut from each block and stained with Harris's acid hematoxylin or Delafield's hematoxylin and counterstained with either aqueous eosin, yellowish or methylene blue.

RESULTS AND DISCUSSION

Seven of 28 inoculated poults (25%) in 5 separate experiments were infected by transfusing into the wing vein 0.75 to 3.0 ml of whole blood obtained from the cecal or mesenteric veins of the donor birds. All of these infections were derived from 3 of 6 donor birds used, the blood of which was inoculated into 16 susceptible poults. No infections resulted from the transfusion of heart or wing vein blood from any of the donor birds.

Microscopic examination of both fresh and stained samples (Wright's or Giemsa stains) of the cecal or mesenteric blood used for transfusion revealed no recognizable forms of *H. meleagridis*.

Lesions produced in all fatal cases of blood-induced blackhead included the

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liver and the lung nearest the site of inoculation in the wing vein. Both lungs were severely caseated and necrotic in 5 of the 7 (71%) infected birds. Kidney lesions were macroscopically visible in 4 of the 7 birds (57%) which became infected through portal blood inoculation. Similar necrotic areas were noted on the spleen of 1 bird, and on the heart of another. Lesions showing similar form and appearance to those in the liver were also found on the pancreas of 1 bird infected by blood transfer.

Only once was a cutaneous lesion found at the site of blood transfusion into the wing veins. Such lesions were described by Tyzzer (1920) and DeVolt and Davis (1936), who produced them by subcutaneous implant of pieces of necrotic liver.

No cecal or intestinal involvement was observed in any of the birds receiving blood transfusions and no abnormality in the cecal droppings was observed in these birds prior to death.

Liver: The liver lesions produced in these birds varied from numerous tiny pinpoint foci to a single massive necrotic area nearly the size of a half dollar. The form and appearance of all lesions were similar to those found in natural outbreaks of blackhead. Passage by cloacal inoculations of fresh triturates prepared from these livers produced blackhead in 8 of 10 (80%) attempts.

Sections of liver revealed numerous small to large well-defined lesions in which clusters of protozoa were seen in relatively clear areas of degenerating liver tissue. These areas were usually near a blood vessel and contained cellular debris, erythrocytes, and sero-cellular exudate. Lymphatic infiltration, multi-nucleated giant cells, heterophils and other inflammatory and phagocytic cells were also seen near the areas of necrotic parenchyma and groups of acidophilic protozoa were found clustered within the periphery of these lesions (see Fig. 1).

Lung: Lung lesions produced by the introduction of blood from the hepatic portal system of a donor bird into the wing vein usually consisted of a firm mass of yellowish caseous material which frequently involved 1/3 to 1/2 of the lung capacity. Smaller nodules of caseous material were often found interspersed among normal lung tissue and were separated at their boundaries by a thick, red, pliable interface of edematous, degenerating tissue and by blood clots.

Microscopic observations of sections of these lung lesions showed generalized areas of coagulation necrosis with alveoli filled with sero-cellular exudate. Hemorrhage and degeneration of blood vessels, bronchial epithelium, and lamina propria were also often observed. Diffuse lymphocytic infiltration and giant cells distended with parasites were seen around these nodules. Many well defined protozoan forms were also found in these areas (see Figs. 3 and 4).

Kidney: Necrotic areas were macroscopically visible in the kidneys of 4 of the fatal cases of blood-induced blackhead. These raised, fibrous, nodules and tumor-like masses often extended to both kidneys and to the surrounding mesenteries and muscle tissues.

Sections of kidney revealed protozoan forms in and around the glomeruli, with evidence of hemorrhage, congestion, and coagulation necrosis. Giant cells and lymphocytic infiltration were observed near the relatively clear areas of tissue degeneration as indicated by the presence of serous exudate.

Spleen: A grayish nodule approximately 2 cm in diameter was seen on the dorsal surface of the spleen of 1 bird infected by transfused cecal blood.

When this organ was sectioned this area showed caseation necrosis and pathology

similar to that seen in the kidney and described above. A few small necrotic foci were seen among other areas of normal tissue but little tissue reaction was observed except in the area of the large nodule. Protozoa were observed in close apposition to blood vessels.

Heart: One blood-induced blackhead transmission resulted in a macroscopically visible grey, fibrous tumor-like accumulation on the caudal tip of the heart.

Microscopic examination of this lesion revealed normal myocardium but definite thickening of the pericardium. A sero-cellular exudate containing many macrophages and vacuoles which may have been formed as the result of the degeneration of protozoa was noted (see Fig. 2).

Proventriculus: Lesions similar to the above-described heart nodule were observed on the dorsal and lateral portions of the proventriculus of 1 infected bird. Photographs were made of these lesions but no tissues were saved from this proventriculus.

Pancreas: One bird which died as a result of blood-induced blackhead also showed circular, depressed areas of necrosis in the pancreas which are characteristic of the lesions of this disease in the liver. Several of these lesions were clearly visible on the surface of the pancreas and extended well into the tissue as they do in liver tissues. Photographs were made of these pancreatic lesions but no sections were prepared.

No infection was produced when 4 birds were inoculated into the wing vein with 1 to 3 ml of whole blood obtained from the wing vein or heart of the donor bird whose cecal blood provided 33% lethal infection.

No blackhead or extraneous disease was noted on sacrifice and autopsy of 21 negative controls. Positive controls kept for each experiment demonstrated the susceptibility of the experimental poults when 12 of the 14 (85%) birds infected by cloacal inoculation of fresh triturate of liver lesions from the donor birds died of typical blackhead. Susceptibility to blackhead was further demonstrated in 2 experiments by the oral administration of approximately 1000 embryonated *Heterakis gallinae* ova. Six of 8 of these infected control turkeys (75%) died of blackhead.

SUMMARY

In 5 separate trials, 7 of 28 (25%) fatal cases of blood-induced blackhead have been produced by inoculating citrated whole blood withdrawn from veins draining the ceca of diseased donor birds into the wing veins of susceptible turkeys. This procedure has produced typical lesions of blackhead in the liver with atypical involvement of lungs, kidneys, heart, spleen, pancreas and proventriculus, but in no instance was the ceca or any portion of the lower intestinal tract involved. *H. meleagridis* was demonstrated in the liver and in these unusual tissue sites as a result of this route of infection but no histomonads were observed from fresh or stained samples of the blood used for inoculation.

The production of typical lesions of *H. meleagridis* in liver and the establishment of the parasites in other organs indicates that this protozoan is capable of entrance and survival in the turkey bloodstream. These results also suggest that this parasite is less fastidious than has been previously indicated by its liver and cecal pathology since it appears that *H. meleagridis* may become established and cause pathology in nearly any type of tissue to which it can gain access. These results therefore

appear to offer indirect evidence of the passage of this protozoan from the ceca to the liver through the hepatic portal system. The liver apparently acts as an efficient filter for these organisms, usually preventing pathology in other suitable tissue sites when infection proceeds by natural routes.

ACKNOWLEDGMENT

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EXPLANATION OF PLATE

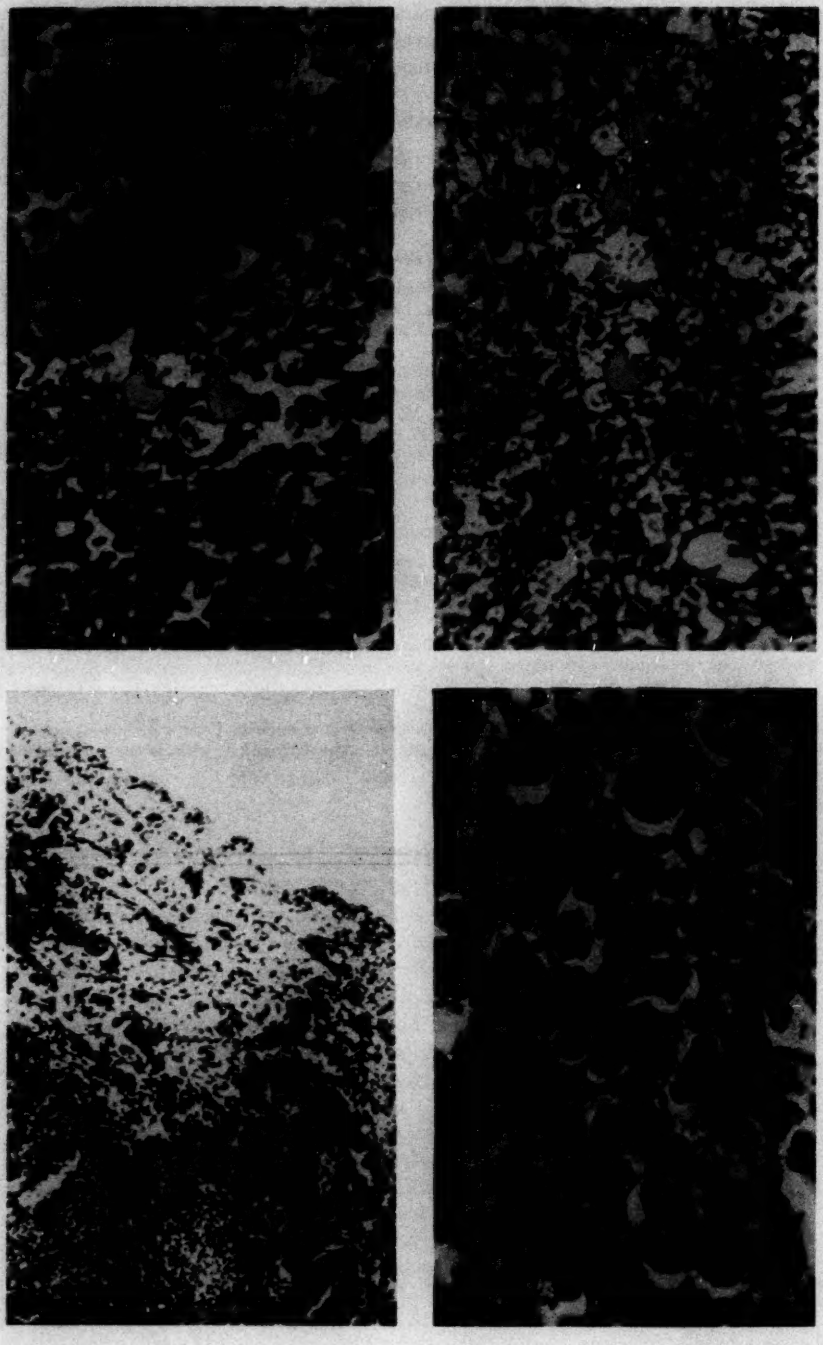
FIGURE 1. Liver. H and E. ×430. Groups of *Histomonas meleagridis* in liver tissue showing an area of degeneration with lymphocytic foci and giant cells with ingested protozoa (arrows). Regenerating tissue may be seen at the upper left and lower right contrasting the typical pathology of the central area.

FIGURE 2. Heart. H and E. ×430. Macrophages clustered in areas of necrotic heart tissues which are swollen with edema and vacuolation.

FIGURE 3. Lung. H and E. ×100. Necrotic degeneration and lumen-formation (containing exudate) between fibrosed pulmonary tissue.

FIGURE 4. Lung. H and E. ×430. Enlargement of portion of Figure 3 showing groups of protozoa in necrotic areas as well as macrophages and inflammatory cells in the surrounding tissue.

PLATE I



GROWTH AND DEVELOPMENT OF *HETERAKIS GALLINAE* IN TURKEYS AND CHICKENS INFECTED WITH *HISTOMONAS MELEAGRIDIS*

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Under natural conditions blackhead in both turkeys and chickens usually results from the ingestion of *Histomonas*-infected eggs of the cecal worm, *Heterakis gallinae*, the unprotected histomonads having difficulty in surviving the passage through the gizzard (Horton-Smith and Long, 1955, 1956; Lund, 1956).

The number and size of the cecal worms recovered at necropsy has been used as a means of approximating the time of infection with *Histomonas*, and also as an indication of the degree of pollution of the source from which the infections were acquired. However, as shown by Baker (1933) and Roberts (1937), there has been considerable disagreement in the literature on the rate of development of these worms in chickens. Both authors attributed at least some of the differences to the effects of blackhead. Almost no data exist which indicate the rate of development of cecal worms in turkeys.

The present study was designed to obtain additional information on the rate of growth of cecal worms in both turkeys and chickens which had had an opportunity to acquire histomoniasis through the ingestion of *Heterakis* eggs.

MATERIALS AND METHODS

Cecal worms, *Heterakis gallinae*, were collected from 17 turkeys which had been on ground recently occupied by other turkeys, some of which had had blackhead and harbored cecal worms. The mature female worms from these 17 turkeys were placed in physiological saline and kept at room temperature (about 72° F) until the eggs capable of embryonating had done so. The eggs were then removed from the bodies of the worms, pooled, and washed in several changes of physiological saline to remove debris. The suspension of eggs in saline was calibrated, and the volume was adjusted so that 1 ml of the agitated suspension contained a calculated 100 embryonated eggs.

Sixty Beltsville Small White poults 7 weeks old and 60 New Hampshire Red chickens 5 weeks old which had been raised under parasite-free conditions were each given orally approximately 100 embryonated *Heterakis* eggs suspended in 1 ml of physiological saline, followed by 1 ml of saline, not containing eggs, to wash out the pipette. Six turkeys and 6 chickens were designated to be killed for study at each of the following intervals subsequent to the administration of the eggs: 10, 14, 17, 21, 28, 35, 42, 50, 56, and 63 days. Birds dying on "kill days" were included among the 6 scheduled for that day, and shortages were absorbed by reducing to 5 birds the number killed in as many of the later periods of observation as necessary. One turkey and 2 chickens were lost due to other causes.

Immediately after the birds were killed, the ceca and livers were examined for lesions and other evidence of histomoniasis, including a search of as many as 4 cecal smears for *Histomonas*. Each cecum was then washed thoroughly to remove the *Heterakis*. All the cecal worms from each bird were collected and counted, and 10 living, intact worms of each sex, selected at random, or as many as were found up to that number in each bird, were measured.

A small auxiliary group of turkeys was used for observations on worm development prior to the 10th day, but these birds contributed no other data to this experiment.

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RESULTS AND DISCUSSION

Incidence of subclinical and clinical blackhead in the test birds

The number of turkeys and chickens harboring subclinical infections of *Histomonas* and the number showing clinical histomoniasis at necropsy are shown in Table I. Detection of *Histomonas* in the cecal contents is always more difficult during the first 2 weeks after infection than it is later. For this reason the results obtained for the period 17 to 63 days after inoculation are considered a better index of total infection than are those obtained from observations made prior to that time. This is particularly true in turkeys where the histomonads may be somewhat slower in getting started than they are in chickens.

TABLE I. *A comparison of the occurrence of* Histomonas *infection and clinical blackhead in turkeys and chickens, each of which received 100 embryonated eggs of* Heterakis gallinae *from the same source.*

		Turkeys		Chickens	
		Number	Percent	Number	Percent
Birds contributing data		59	—	58	—
Blackhead mortality		1	1.7	0	—
Infected with *Histomonas*	10–14 days	5/12*	41.7	7/12*	58.3
	17–63 days	30/47	63.8	33/46	71.6
	10–63 days	35/59	59.3	40/58	69.0
Showing clinical blackhead	10–14 days	2/12	16.7	6/12	50.0
	17–63 days	20/47	42.5	‡	
	10–63 days	22/59	37.3	‡	

* The numerator indicates the number of birds found to be infected; the denominator, the number of birds examined.
‡ Recovery from blackhead is often too complete to permit trustworthy classification.

Histomonas infection among turkeys killed from the 17th to the 63rd day after infection averaged nearly 64%. This represented 1 infection for 157 embryonated eggs fed. Of the chickens studied during this same period, almost 72% were infected. This is considered to represent 1 *Histomonas* infection for each 139 embryonated eggs given. Inasmuch as the eggs given the turkeys and chickens were from the same source, it can be assumed that at least 1 of each 139 embryonated eggs harbored a histomonad.

Worm recovery in birds without clinical blackhead

From 37 turkeys which did not exhibit clinical blackhead, 1,587 heterakids were recovered, an average of 42.6 worms per bird. Thirteen of these 37 birds had subclinical infections of histomonads, and averaged nearly 54 worms per bird, as compared with 37 worms per bird in the 24 turkeys which apparently were free of this protozoan. It is assumed that the individual inocula may have differed somewhat in egg count, and the hatchability of the eggs may have differed somewhat in different birds. The more eggs taken in, and the more that hatch, the greater the chance of *Histomonas* infection, presumably.

Clinical blackhead appears early in chickens and the characteristic tissue responses may be completely obliterated by the 28th day, but *Histomonas* may persist for another 3–6 weeks or longer.

Sixteen chickens not affected with clinical blackhead were studied from 10–21 days after inoculation. These chickens averaged 74 worms per bird. Ten of these

16 birds harbored *Histomonas* and their average worm count was about 76, as compared with an average count of 70 for the 6 birds that did not have histomonads.

In this test, as in others which we have conducted, *Heterakis* recovery was higher in normal chickens than in normal turkeys.

Influence of clinical blackhead on worm recovery

Eleven of the 22 turkeys which showed clinical blackhead had no worms, and the remainder averaged only 22 worms per bird. Thus, it appears that blackhead mild enough to cause less than 2% mortality in turkeys could provoke symptoms sufficient to cause half of the birds to lose all of their worms, and the remainder to lose more than ½ of their worms. Most of this loss occurred during the 3rd and and 4th weeks after infection.

The reduction in numbers of worms occurred even earlier in chickens than was the case in turkeys and it could not be calculated subsequent to the 28th day because recovery was so complete as to make impossible reliable classification of the birds. However, more than ½ of the chickens killed from 28 to 63 days after inoculation had no worms; so it seems that the situation was comparable to that observed with turkeys.

Size of worms in birds without clinical blackhead

Figure 1 illustrates the results obtained by measuring recently removed, living,

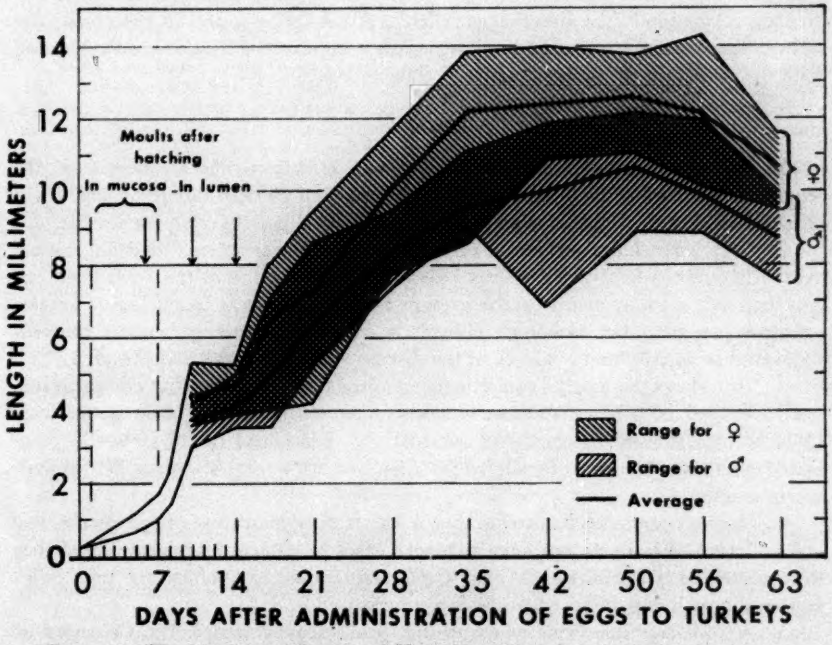

FIGURE 1. The length in millimeters of *Heterakis gallinae* from healthy turkeys at several intervals following the administration of freshly embryonated eggs. Maximum, minimum, and average lengths are shown for each sex.

intact worms of the ages designated, from turkeys that did not show clinical blackhead. A total of 233 females and 200 males 10 to 63 days of age were measured. The maximum, minimum and average size of worms measured at each interval are indicated for each sex. These results have been compared with those obtained from several other experiments in which a total of more than 2000 worms of each sex were measured, and have been found to be representative.

The sizes of the worms grown in chickens not having clinical blackhead were essentially the same as those from turkeys.

Size of worms in birds with clinical blackhead

Thirty-seven female worms grown in turkeys having clinical blackhead were measured, and their lengths averaged 5.6% less than those of females of comparable ages from turkeys not having blackhead. The retardation was consistent, but the value can hardly be regarded as being statistically significant. Male worms from the diseased turkeys showed no consistent retardation.

Dead worms were common in diseased birds, and these worms were smaller, of course. Investigators who may have measured dead worms, or who have fixed the worms before measuring them, must certainly have obtained results differing from ours. Living worms measured before fixation in 70% alcohol or 10% formalin were found to have shrunk 10–20% in length after fixation.

Female worms from chickens with clinical blackhead were 5.1% shorter than worms of comparable age from healthy chickens, but the males were less than 2% shorter. Apparently the situation parallels that for turkeys, and in both hosts the retardation in length probably is of negligible importance, at least when compared with the reduction in numbers, which is considerable.

SUMMARY

1. The rate of growth of *Heterakis gallinae*, as indicated by an increase in the length of the worms, has been studied in turkeys and chickens some of which had clinical blackhead. Rates of growth of worms in the 2 hosts were quite similar.

2. Ceca with clinical evidences of blackhead had fewer worms than did normal ceca. In turkeys half of the diseased ceca had no worms, and the remainder had less than half as many worms as the average for normal ceca. In chickens a similar situation prevailed, but was more difficult to calculate accurately because chickens recovered so rapidly that evidence of the disease was often quickly obliterated.

3. In turkeys the lengths of the female worms from ceca showing clinical blackhead averaged 5.6% less than those of worms from normal ceca. Male worms from diseased ceca showed no consistent retardation. Blackhead in chickens was associated with retardations in female worm length of 5.1%, and less than 2% in male worm length.

4. Worm counts can be used to give a satisfactory indication of egg intake, and soil pollution, if birds do not have advanced cases of clinical blackhead, but if they do, the method is worthless. Worm length can be used to establish the time of infection if only living, intact individuals are measured.

5. *Histomonas* infections which do not provoke macroscopic cecal changes do not influence worm counts or worm lengths, but the prevalence of such infections is a rough indication of soil pollution with *Histomonas*-infected *Heterakis* eggs.

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RESEARCH NOTE

THE OCCURRENCE OF MITES ON MOSQUITOES

In the summer of 1954 the author was using a light trap for following the seasonal distribution of mosquitoes at Itasca State Park, Clearwater County, Minnesota. During the course of this study it became apparent that some species of mosquitoes were heavily parasitized by mites. The mites were larvae of the type frequently found on mosquitoes and presumably are hydrachnids. They cannot be identified in the larval stage at the present time. Some mosquitoes were, however, very heavily parasitized and it was wondered if an attack rate could be calculated for these mosquitoes. The mites on a particular species of mosquito appeared to be all the same and practically no mites could be found free in the light trap.

All *Mansonia perturbans* (Walker) taken on July 5 and 6 were examined and the number of mites recorded; only 24 of the mosquitoes were male. There was, on 420 mosquitoes examined, a total of 572 mites, an average of 1.36 per mosquito. The majority of the mosquitoes examined (70.2%) had no mites at all and the number of mites on an infested mosquito ranged from 1 to 33.

If the mites were randomly distributed among the mosquitoes mathematical description of the situation should be possible. A theoretical frequency distribution was derived from tables of Poisson's exponential expansion (K. Pearson, *Tables for Statisticians and Biometricians*, 1914, Cambridge University Press) for a value of 1.4. This theoretical distribution was then compared with the observed distribution (Table I).

TABLE I. *Frequency distribution of mites on* Mansonia perturbans (*theoretical and actual*).

Number of mites	0	1	2	3	4	5	6	7 or more
Theoretical probability of occurrence	.247	.345	.242	.113	.039	.011	.002	.001
Actual proportion of mosquitoes	.702	.109	.043	.048	.014	.010	.017	.057

The two distributions are quite different so that the hypothesis of random distribution of mites among mosquitoes can be confidently rejected in this case. The reasons for this lack of agreement can only be speculated upon but it might be due to concentrations of mites, to different susceptibilities of individual mosquitoes, or to other reasons.

Of all of the mites counted about 3% were attached to the head, 90% to the thorax (mostly to the anterior part of the neck region), and 7% to the abdomen. A sample of 114 *Aedes vexans* (Meigen) adults taken at the same time showed a total of 4 mites on 3 mosquitoes; all were attached to the posterior part of the thorax.—A. RALPH BARR, *Department of Entomology, University of Kansas, Lawrence, Kansas.*

A NOTE ON *EIMERIA BRAZILIENSIS* TORRES AND RAMOS 1939, AND ITS RELATIONSHIP TO *EIMERIA BÖHMI* SUPPERER 1952

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In a report on the coccidia infesting cattle in Brazil, Torres and Ramos (1939) described the oöcysts of a new species which they called *Eimeria braziliensis*. An interesting feature of many of these oöcysts was the presence of a polar cap, a structure not previously seen in any of the species of coccidian oöcysts recovered from cattle.

In the literature available to the present authors they can find no further references to the occurrence of *E. braziliensis* in other parts of the world. Christensen (1941) recognized 8 distinct species in the course of a detailed survey of the coccidian oöcysts of cattle in Alabama, but did not include *E. braziliensis*. Morgan and Hawkins (1948) recorded its existence but it appears that their source of information was the original description. Lapage (1956) fails to mention its presence in the British Isles and Henning (1956) does not refer to it in describing bovine coccidiosis in South Africa, though he lists most of the coccidian species described from cattle. It should be mentioned, however, that Supperer (1952) described oöcysts with polar caps from the feces of cattle in Austria; this will be considered in more detail later in this paper.

In the course of observations made on cattle feces during the past 3 years in Nigeria, coccidian oöcysts with polar caps have frequently been seen. Critical examination of such oöcysts has shown them to be indistinguishable from *E. braziliensis* and in view of the infrequency of reports on this species details of the present material are now recorded.

MATERIALS AND METHODS

A total of 101 oöcysts recovered from the feces of 5 cattle reared in the vicinity of Vom was examined. The oöcysts were recovered and examined morphologically by the method previously employed at this laboratory (Lee, 1954). On this occasion, however, measurements were made by means of a Leitz hair micrometer and the oil immersion objective. Sporulation times were determined by the technique described by Christensen (1941) except that during the present tests the material was maintained at 27° C.

FINDINGS

The oöcysts were typically ellipsoidal and under oil immersion were yellowish in color with yellowish-grey cytoplasm. The outer layer of the wall was bright yellow, shading to lavender-pink towards the inner surface and there was a distinct micropyle at one pole. The most characteristic feature was the presence of a polar cap at one end overlying the micropyle. The caps were approximately 12 microns wide by 1.5 to 2.0 microns high and showed a tendency to collapse during storage in unprepared feces kept in the refrigerator for a few days. These features are in agreement with those described by Torres and Ramos for *E. braziliensis*.

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The latter authors also described a "tenue portion" of cytoplasm which on contraction of the cytoplasmic mass during the early stages of sporulation adhered to the micropyle, forming a "tenue residual polar body." This was a constant feature of the Vom oöcysts and in Figure 1 an isolated portion of cytoplasm can be seen in the vicinity of the micropyle.

In tests carried out on 12 oöcysts, sporulation was completed in 6 to 7 days.

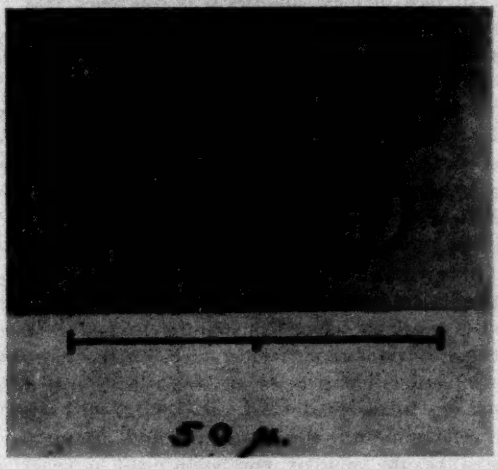

FIGURE 1. Unsporulated oocyst of *E. braziliensis* under oil immersion.

The measurements and other pertinent findings are summarized in Table I and compared with similar data on Torres and Ramos' (1939) oöcysts and on those described by Supperer (1952) as *Eimeria böhmi.*

TABLE I.—*Dimensions and other data relating to* E. braziliensis *and* E. böhmi. (*Measurements in microns.*)

	E. braziliensis Nigerian oöcysts	*E. braziliensis* Torres *et al.* (1939)	*E. bohmi* Supperer (1952)
Mean length	35.7 ± 0.175	37.5	
Range-length	30.7–39.5	34.2–42.7	33.75–42.1
Mean width	25.6 ± 0.134	27.1	
Range width	21.9–29.9	24.2–29.9	24.1–27.7
Mean shape index	0.72	0.74	
Range shape indices	0.63–0.87	0.67–0.83	
Width of polar cap	12.0	8.0–10.0	10.0–12.0
Height of polar cap	1.5–2.0	2–3	2–4
Sporulation time	6–7 days	6 days	12–14 days
No. of oöcytes measured	101	128	62
No. of hosts sampled	5	4	9

DISCUSSION

Though the oöcysts examined are, in general, somewhat smaller than those described by Torres and Ramos, the difference is slight for coccidian oöcysts, and they should be considered as *Eimeria braziliensis.*

Supperer (1952) studying the coccidia of cattle in Austria found oöcysts with polar caps and he considered that they were, therefore, different from all known oöcysts of cattle and described them as *Eimeria böhmi* n. sp.

In Table I, it will be seen that there is no significant difference between the dimensions of the Austrian and the Brazilian oöcysts. Two sketches of *E. böhmi* which are included in Supperer's text clearly show a distinct body behind the micropyle in the same position as the residual polar body seen in the original and the Nigerian specimens of *E. braziliensis*. These residual polar bodies have not been seen in the other 9 or 10 distinct oöcyst species known to occur in the feces of Nigerian cattle. In view of these findings it is concluded that *E. böhmi* oöcysts are morphologically indistinguishable from those of *E. braziliensis*.

In one respect, however, Supperer's oöcysts differ markedly from *E. braziliensis*. Torres and Ramos and the Vom specimens sporulated in 6 to 7 days, whereas *E. böhmi* required 12 to 14 days. In determining the sporulation time Supperer also used, without modification, Christensen's (1941) technique which is carried out at room temperature. In a personal communication, Supperer (1957) states that the room temperature at the time his tests on *E. böhmi* were made was about 20° C, which is some 7°C lower than the temperature at which the tests on *E. braziliensis* at Vom were conducted.

Christensen (1939) showed that the sporulation time of *E. arloingi* of sheep is accelerated by gross increases in temperature. No work appears to have been done on the effect of relatively minor temperature changes within the ranges likely to be encountered at different room temperatures on the sporulation time of the coccidian oöcysts of cattle. Nevertheless, it seems likely that differences in room temperatures obtaining in temperate and sub-tropical or tropical climates could be sufficient to affect significantly the sporulation time of coccidian oöcysts. It is, therefore, suggested that the value of sporulation times as an aid to the classification of coccidian oöcysts would be enhanced by conducting the determinations at a constant, stated temperature. Until more information is available on this subject, however, *E. böhmi* may claim validity on the evidence of its prolonged sporulation time.

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THE LIFE HISTORIES OF SOME HAWAIIAN HETEROPHYID TREMATODES*

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INTRODUCTION

In March 1956 the author was flown to Hawaii by the United States Navy. With the assistance of Dr. and Mrs. A. H. Banner and Ralph Hinegardner, several thousand snails, *Stenomelania newcombi* Lea and *Tarebia granifera* Lam., were collected in ditches surrounding taro patches in Kaneohe, Oahu. They were taken to the Hancock Foundation, University of Southern California, for examination and life cycle experiments which revealed that 4 species of heterophyid trematodes, *Centrocestus formosanus* (Nishigori, 1924), *Haplorchis taichui* (Nishigori, 1924), *H. yokogawai* (Katsuta, 1932), and *Stellantchasmus falcatus* Onji and Nishio, 1924, use one or both of these snails as first intermediate hosts. The work was completed at the Marine Laboratory, University of Hawaii.

Very little information has been published on Hawaiian heterophyids even though the infections are common in *S. newcombi* and *T. granifera*, at least one of the second intermediate hosts, *Mugil cephalus*, is eaten raw by humans, and 2 cases of human infection have been reported (Alicata and Schattenburg, 1938; Glover and Alicata, 1957).

Centrocestus formosanus was described by Nishigori (1924a) and placed by him in the genus *Stamnosoma* which was erected by Tanabe (1922) but is synonymous with *Centrocestus* Looss, 1899 (Chapin, 1926). Nishigori stated that the first intermediate host was a species of *Melania* into which the miracidium penetrated and developed into a redia. He did not mention a sporocyst stage. The rediae produced cercariae which encysted in the gills of fresh water fish. He listed the following second intermediate hosts: *Carassius auratus* (Linnaeus), *Clarias fuscus* (Lacépède), *Channa formosana* (Jordan and Evermann), *Pseudorasbora parva* (Schlegel), *Rhodeus ocellatus* (Kner), *Gambusia affinis* (Baird and Girard), *Polyacanthus operculatus* (Linnaeus), *Ctenopharyngodon idellus* (C. and V.), *Ophiocephalus tadianus* (Jordan and Evermann), *Misgurnus anguillicaudatus* (Cantor), *Parasilurus asotus* (L.), *Zacco platypus* (T. and S.) and *Cyprinus carpio* (L.). He found the night heron, *Nycticorax nycticorax* (L.) to be a natural definitive host and obtained experimental infections in several species of laboratory animals and in man. Vazquez-Colet and Africa (1938, 1939, 1940) added to the list of second intermediate hosts, *Ophiocephalus striatus* Bloch, *Glossogobius giurnus* Buchanan-Hamilton, *Therapon plumbeus* (Kner), *Anabas testudineus* (Bloch)

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and *Hemiramphus dussumieri* Cuvier and Valenciennes from Phillippine waters. Chen (1942, 1948) provided additional information on the morphology and life cycle of this parasite including the fact that a frog, *Rana limnocharis* Boie and a toad, *Bufo melanostictus* Schneider, could serve as second intermediate hosts. However, attempts to parasitize adult frogs were negative. Therefore, he concluded that the tadpole stage must become infected (Chen, 1948). He found that *Carassius auratus, Macropodus opercularis* (L.), *Puntius semifasciolatus* (Gunther), *Limia caudofasciata* Regon and *Misgurnus anguillicaudatus* could serve as second intermediate hosts in China.

Haplorchis taichui was described by Nishigori (1924b) and placed in the genus *Monorchotrema*. *Monorchotrema* was declared a synonym of *Haplorchis* Looss, 1899 by Witenberg (1930). The life cycle of *Haplorchis taichui* was first outlined by Nishigori (1924b), then traced in detail by Faust and Nishigori (1926). Hsu (1950, 1951) also made a detailed study of the life cycle of this and related species and showed that Faust and Nishigori had incorrectly described the tail fins of the cercaria. The first intermediate hosts were species of the snail genus *Melania, M. obliquegranosa* (acc. to Faust and Nishigori) and *M. tuberculata chinensis* (acc. to Hsu). Second intermediate hosts included: *Cyprinus carpio, Carassius auratus, Zacco platypus, Pseudorasbora parva, Rhodeus ocellatus, Gambusia affinis, Ctenopharyngodon idellus* (acc. to Faust and Nishigori, 1926); *Varicorhinus* sp., *Tilapia simonis, Barbus canis* and *Gambusia affinis* (acc. to Witenberg, 1929); *Ophiocephalus striatus* (acc. to Vazquez-Colet and Africa, 1939); and *Oryzia latipes* and *Macropodus opercularis* (acc. to Hsu, 1951). Definitive hosts included: birds, *Nyctycorax nyctycorax* (acc. to Nishigori, 1924b), cattle egrets (Africa, 1938), *Larus* sp. (Witenberg, 1929), ducklings (Hsu, 1951) and mammals, cats and/or dogs (Faust and Nishigori, 1926; Witenberg, 1929; Africa and Garcia, 1935; Africa, 1938; Kuntz and Chandler, 1956); fox, *Vulpes vulpes* (acc. to Kuntz and Chandler, 1956); and man (Faust and Nishigori, 1926; Africa and Garcia, 1935; Africa, 1938; and Africa, de Leon and Garcia, 1935ab, 1936ab, 1937, 1940). This species has been reported from China (Chen, 1936), Egypt, (Kuntz and Chandler, 1956), Formosa (Faust and Nishigori, 1926), India (Bhalerao, 1936), Israel (Witenberg, 1929), and the Philippines (Vazquez-Colet and Africa, 1939).

Haplorchis yokogawai was described by Katsuta (1932a) from Formosa. He found the mullet to be a second intermediate host. Chen (1936) added further details of morphology especially of the gonotyl of specimens collected in China. Africa and Garcia (1935) and Africa (1938) reported this species from man in the Philippines. Chen (1936) reported this species from China and Bhalerao (1936) found it in India. Vazquez-Colet and Africa (1938, 1940) found that the fish, *Mugil dussumieri, Hemiramphus georgi, Ambassis buruensis, Arius manilensis, Clarias batrachus, Gerris kapas* and *Ophiocephalus striatus* served as second intermediate hosts to *H. yokogawai* in the Philippines. Kuntz and Chandler (1956) reported this species from the dog, fox, and osprey in Egypt.

Stellantchasmus falcatus was first described by Onji and Nishio (1924) from the small intestine of amidori (a bird), dogs and cats in Japan. They found the metacercariae of this species in the muscle of the fish, *Mugil* and *Liza*. The genus *Liza* has been placed in the synonymy of *Chelon* by Schultz *et al.* (1953). Witenberg (1929) found this trematode in dogs and cats in Palestine but called it *Diorchitrema pseudocirrata* n. g., n. sp. He, too, found the metacercariae in *Mugil*.

Africa and Garcia (1935) were the first to report this species from man. Vazquez-Colet and Africa (1938, 1939, and 1940) found the metacercariae in *Anabas testudinalis* and *Mugil dussumieri* in the Philippines. Alicata and Schattenburg (1938) found this worm in man in Hawaii and stated that *Diorchitrema pseudocirrata* was a synonym of *Stellantchasmus falcatus*. Glover and Alicata (1957) reported another infection in a Hawaiian. Other species assigned to the genus *Stellantchasmus*, *S. formosanus* Katsuta, 1931, which experimentally parasitized man, dogs, cats, and mice to which mullet had been fed and *S. amplicoecalis* Katsuta, 1932b, from dogs, cats and mice, were considered synonyms of *S. falcatus* by Chen (1951). Kuntz and Chandler (1956) reported *S. falcatus* as common in Egyptian cats.

MATERIAL AND METHODS

Infected *Stenomelania newcombi* and *Tarebia granifera* were isolated in finger bowls. Emerged cercariae were studied alive and as fixed and stained whole mounts. Cercariae were fixed without pressure by forcibly ejecting them into cold Bouin's or Heidenhain solution. Earlier larval stages and percentages of infection were obtained by crushing snails. Some of the mullet, *Mugil cephalus*, exposed to cercariae were collected near San Felipe, Baja California. No previous infections were found in any of them. Goldfish, *Carassius auratus* (L.), were purchased from aquarium shops. *Gambusia affinis*, *Kuhlia sandvicensis*, *Lebistes reticulata*, *Tilapia mossambica*, and *Clarias fuscus* were also exposed to cercariae. *Kuhlia sandvicensis* were collected in salt water (Kaneohe Bay) and gradually adapted to fresh water, and the others (except *Clarias fuscus*, Chinese catfish, kindly donated by the University of Hawaii Aquarium) in fresh water ponds or streams of Coconut Island and Oahu. Hatchery-raised chicks and laboratory-raised cats and rats were used as experimental definitive hosts. Larval and adult specimens were fixed in cold Bouin's or Heidenhain solution, stained with Mayer's paracarmine or Celestin blue-B and mounted in Permount or H-S-R Mounting Medium. Serial sections were stained with hematoxylin and triosin.

All measurements are in millimeters unless otherwise stated.

OBSERVATIONS AND DISCUSSION

The parasites described in this paper develop in one or both of the snails, *Stenomelania newcombi* and *Tarebia granifera* in Hawaii. In all of the taro patch ditches examined to date *T. granifera* far outnumbers *S. newcombi*. The percentages of infection vary with the size of the snails and the locality in which they are collected. With little seasonal change and few migratory birds, the percentages of infection probably do not change during the year to the degree found in some regions (Martin, 1955). A total of 419, 201 *S. newcombi* and 218 *T. granifera*, were collected on June 5, 1957, crushed and examined. Of the *S. newcombi*, 16.9% were negative, 38.6% harbored *Stellantchasmus falcatus*, 26.8% *Centrocestus formosanus*, 16.4% *Haplorchis yokogawai* and 1.5% a megalurous type of cercaria. No multiple infections were found. Of the *T. granifera*, 63.8% were negative, 21.6% had *S. falcatus*, 7.3% *H. taichui*, and 7.3% the megalurous cercaria. From these findings it appears that *Haplorchis taichui* will not develop in *Stenomelania newcombi* and *Tarebia granifera* will not serve as host to *Centrocestus formosanus* and *Haplorchis yokogawai*. *T. granifera* seems to be a more favorable host to the megalurous cercaria than *S. newcombi*. Both snails serve as hosts to *S. falcatus*, the most frequent infection. The 419 snails mentioned above were selected for size, the *S. newcombi* at least 2.5 cm in length and the *T. granifera* at least 1.5 cm. In both species there is a directly proportionate relation between the number of infections and the size of the snails. For example, 80 *T. granifera*, at least 10 but less than 15 mm long, were 82.5% negative, 8.75% with *S. falcatus* and 8.75% had *Haplorchis taichui*. Com-

parable results were obtained with the small *S. newcombi*. The small snails have a higher percentage of negative individuals. The variation in infection percentages in different localities will be the subject of another paper.

Centrocestus formosanus (Nishigori, 1924)
(Figs. 1–5)

Approximately 27% of the larger *Stenomelania newcombi* collected were infected with *Centrocestus formosanus*. In the mantle tissue near the gills slender, elongate sporocysts were found in some of the snails. When straightened, some of the sporocysts were 1 cm in length. Young sporocysts are solid but older ones have a core of cells at least partially separated from the wall (Fig. 1). Clusters of nuclei within the core form the rediae which migrate to and develop within the digestive gland of the snail. Young rediae are heavily nucleated throughout (Fig. 3) but in older rediae most of the nuclei are grouped into germ balls. The rediae bear spines and bristles at their anterior ends. Measurements of 24 fixed and stained rediae containing only immature cercariae are: length 0.29–0.45, av. 0.37; width 0.084–0.126, av. 0.10; and pharynx 0.011–0.016, av. 0.014 long and 0.011–0.019, av. 0.015 wide. Only 1 generation of rediae was observed.

The cercariae are biocellate with a double left ocellus and a single right. The anterior ¼th of the body is spined. There are 9 prominent spines immediately anterior to the mouth and a few fine bristles extend from the sides of the body. Light-brown granules are scattered throughout the body. Fourteen penetration glands have their cell bodies located between the pharynx and the excretory bladder. Their ducts pass anteriad to terminate in 4 bundles of 3–4–4–3 near the preoral spines. Cystogenous glands are laterally arranged between the pharynx level and the posterior end of the body. The oral sucker is well developed but the acetabulum is rudimentary. The digestive tract is incomplete consisting of a short prepharynx, a spherical to pyriform pharynx; a short esophagus and a rudimentary intestine. The genital primordium consists of a mass of cells immediately anterior to the excretory bladder. The excretory bladder is thick-walled and varies from a flattened sack to an X-shape. The main excretory tubes arise from the anterolateral portions of the bladder and proceed anteriorly in a somewhat contorted fashion to mid-body level where they divide into anterior and posterior branches. Each branch bears 2 pairs of flame cells. The flame cell pattern is expressed by the formula $2[(2+2)+(2+2)]=16$. The tail is slender and bears a weakly developed dorsoventral fin. The fin extends over approximately the posterior ¾ths of the tail although it does not extend forward ventrally quite to the level of the dorsal starting point. A few nuclei are clustered near each end of the tail. Measurements of 25 fixed and stained specimens are: body length 0.047–0.122, av. 0.088; body width 0.026–0.071; av. 0.05; oral sucker length 0.018–0.026, av. 0.023; oral sucker width 0.015–0.023, av. 0.018; prepharynx length 0.001–0.007, av. 0.004; pharynx length 0.005–0.009, av. 0.008; pharynx width 0.006–0.008, av. 0.008; tail length 0.062–0.098, av. 0.078; maximum tail width 0.011–0.015, av. 0.013.

The cercariae leave the snail and swim slowly in the water. If they come in contact with the gills of a suitable host they quickly shed their tails, penetrate and encyst. Fish with thick gills are more favorable hosts than those with slender gill filaments. *Mugil cephalus* and *Tilapia mossambica* have slender gill filaments and only extremely light natural infections have been found in the former, and the latter did

not become infected even after exposure to hundreds of cercariae. Experimental infections were produced by exposing goldfish (Fig. 3), *Gambusia affinis* (Baird and Girard), and *Xiphophorus helleri* Heckel. Natural infections occur in *Gambusia affinis* and *Xiphophorus helleri* from the taro patch ditches where the snails were collected. Forty *Gambusia* and 3 *Xiphophorus* from these ditches were examined. They varied from 1 to 3 inches in length. Only 3 of the *Gambusia* were negative. The remaining 37 had from 3 to 678 metacercariae per fish in the gills. They averaged 59.9 metacercariae per fish. The fish having 678 was only 2½ inches long. The 3 *Xiphophorus* contained from 250 to 500 metacercariae and averaged 411 per fish. The heavy natural infections probably have been accumulated over some period of time because a single experimental exposure to large numbers of cercariae may result in the death of the fish. The metacercarial cysts are oval with relatively thick walls. In fully developed metacercariae the eyespots break down but the pigment granules are still evident, the circumoral crown of usually 32 spines arranged in 2 rows and characteristic of the adult are developed, the digestive tract is complete and the ceca are filled with disc-shaped concretions, the excretory bladder becomes X- or I-shaped and is filled with globular concretions differing chemically from those of the intestinal ceca (the subject of another paper), and the reproductive organs are discernible.

Experimentally infected fish were fed to hatchery-raised chicks, white mice, white rats, and cats. Although all of these animals were positive, the rats and cats were more favorable hosts in that a greater percentage of the metacercariae fed became adult and more eggs were present in the uteri than in those developing especially in the chick. The adult *Centrocestus formosanus* were localized in the anterior portion of the small intestine. One rat fed several hundred metacercariae on May 28, 1956 was autopsied June 27, 1956 with the following results: 14 worms in the first 3 inches of the small intestine, 3 in the second, 3 in the third, 3 in the fourth, 1 in the fifth, 1 in the sixth; and the remainder of the intestine was negative. A cat fed 4 infected goldfish July 23, 1956 and examined August 6, 1956 had 148 worms in the first 6 inches of the small intestine, 157 in the second, 12 in the third; and the remainder of the intestine negative. Another cat fed 1813 metacercariae on April 3, 1957 and examined April 10, 1957 had 191 worms in the first 3 inches of the small intestine, 347 in the second, 113 in the third, 17 in the fourth, 5 in the fifth; and the rest of the intestine was negative.

A good description of the adult worm has been given by Chen (1942) who found rats and cats to be natural hosts in China. He indicated that man might become parasitized by eating partially cooked frogs. Natural hosts of *Centrocestus formosanus* in Hawaii include the night heron, *Nycticorax nycticorax*, rats and probably occasionally cats and dogs.

In the present description of the life cycle of *C. formosanus* certain findings are new and some vary from previous descriptions. The sporocyst stage has not been previously described. Chen (1948) showed only 4 flame cells in the cercaria but 16 were found by the present author. The latter number agrees with the flame cell count given by Yamaguti (1938) for the cercaria of *Centrocestus armatus* (Tanabe). Yamaguti showed only 4 penetration glands on each side of the body in *C. armatus* but this probably is incorrect. Chen (1948) reported a variation of 30-36 circumoral spines in the adult *C. formosanus*. In the present work, a variation of 31–34 circumoral spines was found in 24 worms. All but 5 of these had 32 spines.

Stenomelania newcombi Lea is a new first intermediate host. New second intermediate hosts are: *Kuhlia sandvicensis* (Steindachner), *Mugil cephalus* (Baird and Girard) and *Xiphophorus helleri* Heckel. This is the first report of *Centrocestus formosanus* in Hawaii, where it has been introduced probably from the Orient. Previously it has been reported from Formosa, Japan, China, and the Philippines.

Stellantchasmus falcatus Onji and Nishio, 1924
(Figs. 6–13)

Approximately 39% of the *Stenomelania newcombi* and 22% of the *Tarebia granifera* collected were infected with *Stellantchasmus falcatus*. Two generations of rediae developed in the digestive glands of these parasitized snails. Rediae vary from almost spherical to slender and elongate. The mouth opens into a small, nearly spherical pharynx which is connected to the saccular gut. In young rediae the gut may be ¾ths the body length but in older specimens it is proportionately much shorter. There is a birth pore near the anterior end. The redial wall contains yellow globules. Measurements of 24 rediae that contained only germ balls are: length 0.112–0.42, av. 0.234; width 0.056–0.112, av. 0.087; pharynx length 0.015–0.022, av. 0.018; pharynx width 0.015–0.022, av. 0.019.

The cercariae are ocellate and pleurolophocercous. The left eyespot is double and the right single. Brown granules are scattered through the body. The body cuticle is covered with spines arranged in a quincunx and decrease in size and number posteriorly. A few lateral bristles project from the body surface. The oral sucker is well developed but the acetabulum is rudimentary. Anterior to the mouth is a spined area consisting of 3 rows, with usually 5 elongate spines whose points project slightly into the mouth in the first row and 7 to 10 shorter spines in each of the other rows. The digestive tract includes a short prepharynx, small pharynx and rudimentary intestine. Fourteen penetration glands are arranged in 2 rows. The cell bodies of these glands extend from a short distance posterior to the pharynx to near the posterior end of the body. Their ducts pass anteriad and divide into bundles of 3–4–4–3 near the anterior end of the body. Cystogenous glands are laterally arranged from a little behind the eyespots to near the posterior end of the body. The thick-walled excretory bladder varies from the simple saccular to V-, Y-, or X-shaped. The main collecting tubes proceed anteriad from the anterolateral margins of the bladder to about the mid-body level where they divide into anterior and posterior branches each of which bears 3 groups of 3 flame cells each. The flame cell formula is $2[(3+3+3)+(3+3+3)]=36$. An exit tube from the bladder extends a short distance into the tail and divides into 2 branches which open laterally. The cuticle of the anterior 2/5ths of the tail is swollen and superficially annulated. A fin begins dorsally near the body then extends posteriorly around the tail tip and forward for about 2/5 ths of the tail length. Measurements of 25 fixed and stained cercariae are: body length 0.055–0.116, av. 0.091; maximum body width 0.034–0.064, av. 0.047; oral sucker length 0.018–0.023, av. 0.021; oral sucker width 0.016–0.02, av. 0.018; prepharynx length 0.003–0.011, av. 0.007; pharynx length 0.004–0.009, av. 0.007; pharynx width 0.006–0.009, av. 0.007; tail length 0.24–0.37, av. 0.32; maximum tail width 0.011–0.02, av. 0.017.

When the cercariae come in contact with the fish, *Mugil cephalus*, they quickly shed their tails and penetrate. Encystment occurs principally in the skeletal muscles. *Mugil cephalus* from the Gulf of California were exposed to cercariae and

extremely heavy infections resulted. The metacercarial cysts are oval to nearly spherical. Young metacercariae contain considerable pigment which, with the exception of granules from the eyespots, disappears as the metacercariae become older. Fully developed cysts are approximately 0.30 long and 0.28 wide. The cyst wall is composed of 2 layers, the outer granular and the inner clear. Changes from the cercarial stage include the completion of the digestive tract with the ceca filled with disc-shaped concretions, the development of the gonotyl (acetabulum of other authors) enclosed in a ventrogenital sac, the development of the testes, ovary and reproductive ducts, and the accumulation of concretions in the excretory bladder.

Natural infections have been found in *Mugil cephalus* collected in Kaneohe Bay. Every one of 25 fish examined was heavily parasitized, i.e. had several hundred cysts per fish in the muscles. Even the eye muscles may each contain several metacercariae. These fish probably became parasitized in fresh or possibly in slightly brackish water. In view of this infection rate it is surprising that more cases of human infection have not been reported. It is my opinion that if a survey were made of Hawaiians who eat raw mullet most of them would be found to harbor *S. falcatus*.

M. cephalus from the Gulf of California were exposed to large numbers of cercariae April 23–26, 1956 and were fed to 2 cats on May 4, 22, 29 and June 9 and 11, 1956. One cat was autopsied June 12, 1956 with the following results: the anterior 6 inches of the small intestine contained only a few worms, the second, third and fourth 6-inch lengths contained hundreds of worms each, the fifth and sixth had fewer and the remainder of the intestine was negative. Over fourteen hundred *S. falcatus* were recovered. The other cat examined July 25, 1956 presented much the same picture except that worms first appeared in the fourth 6 inches and were present in large numbers from there on through the eighth 6 inches. The remainder of the intestine was negative.

Measurements of 25 mounted specimens are: body length 0.257–0.387, av. 0.321; maximum body width 0.136–0.214, av. 0.176; oral sucker diameter 0.037–0.053, av. 0.045; prepharynx length 0–0.015, av. 0.004; pharynx length 0.025–0.04, av. 0.031; pharynx width 0.019–0.028, av. 0.024; esophagus length 0.031–0.081, av. 0.055; gonotyl length 0.028–0.038, av. 0.032; gonotyl width 0.028–0.035, av. 0.031; testes length 0.037–0.087, av. 0.058; testes width 0.034–0.068, av. 0.046; ovary length 0.028–0.043, av. 0.031; egg length 0.019–0.022, av. 0.02; and egg width 0.010–0.012, av. 0.011.

The body sizes of these worms are less than those given by other authors. They are nearest to those given by Katsuta (1931) for *S. formosanus*. These discrepancies probably are due to differences in age and/or fixation. The oldest of my specimens had been in the definitive host for 39 days. The ceca in these specimens usually reached to or near the anterior border of the testes whereas Chen (1951) described their extent as only to the lower level of the ovary. The ceca have relatively thick walls. The extent of the vitellaria from the ovarian level to near the posterior end of the body is in agreement with Chen (1951) but Witenberg (1929) showed them only in the testicular area. If this yolk distribution is constant in the Palestine material it may warrant raising Witenberg's specimen from synonymy to the status of a distinct species. Further study of the Palestine material is needed.

Other authors have called the muscular organ found in the ventro-genital sac, an

acetabulum. However, this cup-shaped structure has 2 opposed knobs that are covered with short, blunt spines and the lining of the cup bears scattered spines (Figs. 11–12). It seems to be comparable to the gonotyl of other species. Opposite the gonotyl and ventral to the terminations of the genital ducts there is a fleshy pad which may be a degenerate acetabulum.

Witenberg (1929) stated that the sperm expulsor had very thick walls in which spiral fibers were clearly seen. Chen (1951) stated that the expulsor wall was heavily chitinized. To my knowledge no specific tests for chitin in the expulsors of these worms have been reported. Spiral fibers were seen in the sperm expulsors of my specimens. They look like muscle fibers but no contraction was observed.

Price (1940) considered the genera *Stellantchasmus* and *Procerovum* to be closely related. However, the cercaria of *Procerovum* described by Hsu (1951) is quite different from that of *Stellantchasmus* and indicates that *Procerovum* is more closely related to the haplorchids than to *Stellantchasmus*.

Haplorchis taichui (Nishigori, 1924)
(Figs. 17–19, 21–23)

Approximately 7% of the *Tarebia granifera* collected were infected with *Haplorchis taichui*. Two generations of rediae develop in the digestive gland of these snails. The rediae are slender and elongate. The pharynx is small and spherical or nearly so and the gut is short. Measurements of 15 stained and mounted specimens are: length 0.264–.496, av. 0.408; maximum width 0.05–.084, av. 0.064; pharynx diam. 0.016–.022, av. 0.019.

The cercariae are biocellate with a single ocellus on the right side and a double one on the left. Intercellular brown granules are present from the mid oral-sucker-level to the posterior end of the body. The anterior part of the body is spined and there are a few bristles which extend laterally from the body surface. There is a preoral spined area consisting of usually 4 spines in the row nearest the mouth and 2 rows of 5 to 8 spines in each of the others. The mouth is surrounded by a cuticularized ring. There are 7 penetration glands on each side of the body. The cell bodies of these glands are lobed. They extend from a level just posterior to the pharynx to the posterior end of the body. Their ducts pass anteriad in 2 bundles which divide into a 3–4–4–3 arrangement at their terminations near the mouth. Cystogenous glands containing large granules are laterally arranged from the level of the pharynx to the posterior end of the body. The digestive tract consists of a well developed oral sucker, a short prepharynx, small pharynx and a rudimentary gut. A genital primordium is located immediately anterior to the excretory bladder. The excretory bladder is thick-walled and saccular. The flame cells are exceedingly difficult to observe because of body pigment, lack of contrast between them and surrounding cells and weakly beating cilia. Seven were seen on each side of the body but their capillary tubes could not be traced; 6 of these were in the anterior half of the body. An exit tube from the bladder extends a short distance into the tail where it divides into 2 branches which open laterally. The tail is elongate and slender and is set in a socket of the body. Its anterior third bears lateral fins, near the posterior ends of which a dorsoventral fin begins, extends posteriad around the tip of the tail and then anteriad ventrally to near the middle of the tail. Beneath the tail cuticle there is a cortex of longitudinal muscle fibers. Nuclei are distributed irregularly in the tail medulla.

Measurements of 25 stained and mounted cercariae are: body length 0.089–.124, av. 0.106; maximum body thickness (most specimens were viewed from the side) 0.031–.043, av. 0.035; oral sucker length 0.019–.025, av. 0.021; oral sucker width 0.017–0.02, av. 0.019; pharynx approximately 0.007 long and 0.006 wide; tail length 0.29–.35, av. 0.32; maximum tail thickness 0.016–.022, av. 0.017.

The cercariae leave the snail and swim about in the water. They are positively phototropic. Experimental infections were established in *Gambusia affinis, Mollienesia formosus* and in the goldfish, *Carassius auratus.* Natural infections have been found in *Gambusia affinis, Mollienesia formosus,* and rarely in *Mugil cephalus* collected in taro patch ditches at Kaneohe. The cercariae shed their tails, penetrate and encyst especially at the base of the tail and occasionally in the soft tissue around the mouth and pharynx. They sometimes encyst in the caudal fin of the goldfish.

The metacercarial size varies with age. Recently encysted metacercariae were approximately 0.14 long and 0.11 wide. Fully developed metacercariae were approximately 0.23 long and 0.16 wide. As the metacercaria ages it loses some of its body pigment but the eyespots are still visible, the body spines increase in size, the penetration glands disappear, many tubular, unicellular glands develop in the anterior part of the body especially around the oral sucker, the ceca develop and extend toward but do not reach the posterior end of the excretory bladder and become filled with disc-shaped concretions, the excretory bladder increases in size and becomes filled with globular concretions, the flame cells and excretory tubes become more prominent, the testis and ovary develop and the gonotyl with its armature becomes fully developed.

Adult worms were recovered from the small intestines of cats, some of which were fed experimentally infected *Gambusia* and others naturally infected *Gambusia.* They were located between the villi mainly in the first 24 inches of the small intestine. The major changes from the metacercarial stage include the development of vitellaria, sperm, and eggs. The gonotyl is pyriform with a cavity opening at its distal end. A semi-circle of 12 to 18 crescent-shaped bars is located on one side of the distal portion. On the inner surface of the gonotyl, adjacent to the bars, is a triangular patch of rods and spines (Fig. 23). The cecal walls are thick. Remnants of eyespots are present. The flame cell formula is $2[(2+2+2)+(2+2+2)]=24$.

Measurements of 20 stained and mounted, egg-bearing specimens are: body length 0.201–.353, av. 0.28; maximum body width 0.155–.263, av. 0.21; oral sucker diameter 0.025–.053, av. 0.037; prepharynx absent to very short; pharynx length 0.025–.037, av. 0.031; pharynx width 0.019–.028, av. 0.023; esophagus length 0.053–.087, av. 0.067; testis width 0.053–.074, av. 0.062; testis length 0.053–.087, av. 0.067; gonotyl length 0.034–.043, av. 0.039; gonotyl width 0.028–.037, av. 0.034; egg length 0.023–.026, av. 0.024; egg width 0.011–.014, av. 0.012.

Hsu (1951) stated that there are 2 flame cells on each side of the body in the cercaria of *H. taichui.* Those found by him are merely the ones most easily seen. As was stated previously, at least 7 flame cells are present on each side of the body of the cercaria. In the metacercariae and adults the flame cells and the excretory tubes are much more evident than in the cercariae. It is believed by the author that the excretory formula $2[(2+2+2)+(2+2+2)]=24$ applies to the cercaria as well as to the metacercaria and adult. The same grouping of flame cells in pairs but with only 4 pairs on each side of the body was found in the related species

Euhaplorchis californiensis by Martin (1950). Hsu (1951) also stated that the cercaria of *H. taichui* was among the smallest and the cercaria of *H. pumilio* the largest of the 5 heterophyid cercariae he described. However, if the cercaria of *H. taichui* (his figure 26) had been drawn to the same scale as the cercaria of *H. pumilio* (his figure 27) it would actually be larger than the latter.

This is the first report of *Haplorchis taichui* from Hawaii. Like *Stallantchasmus falcatus,* this species is widely distributed, having been reported previously from China, Egypt, Formosa, India, Israel, and the Philippines. Since it has been associated with human fatalities in the Philippines, it constitutes a health hazard in Hawaii.

Haplorchis yokogawai (Katsuta, 1932)
(Figs. 14–16, 20, 24)

Approximately 16% of the larger *Stenomelania newcombi* collected were infected with this species. Two generations of rediae develop in the digestive gland of the snail. The rediae are slender and elongate to saccular (Fig. 20). The pharynx is small and the gut short. Measurements of 10 rediae that contained only germ balls are: length 0.192–.465, av. 0.304; maximum width 0.046–.081, av. 0.067; pharynx length 0.019–.025, av. 0.022; pharynx width 0.0186–.025, av. 0.019.

The cercariae are similar to those of *H. taichui* in general morphology. However, in fixed specimens, on the average the body is shorter and less pigmented and the tail is longer than in the cercariae of *H. taichui.* In the first row of preoral spines *H. yokogawai* usually has 6 and *H. taichui* 4. Measurements of 20 stained and mounted specimens are: body length 0.068–.096, av. 0.082; maximum body thickness 0.037–.05, av. 0.041; oral sucker diam. 0.018–.023, av. 0.021; pharynx diam. approximately 0.009; tail length 0.310–.353, av. 0.331; maximum tail thickness 0.015–.021, av. 0.017.

A Chinese catfish, *Clarias fuscus,* was exposed to hundreds of cercariae for a period of 25 days. It was then cut into pieces and fed to 2 cats. The other catfish retained as a control was carefully examined and no metacercariae were found. One cat was autopsied 10 days after the experimental feeding and hundreds of *Haplorchis yokogawai* were found in the first half of the small intestine. The second cat was autopsied 14 days after the experimental feeding and the small intestine was cut into 6-inch segments and examined. Seven *H. yokogawai* were found in the first segment and over 100 in each of the second, third, fourth, fifth and sixth. The remainder of the intestine was negative.

The 10-day-old worms from experimentally infected cats contain only a few eggs each and are smaller than the 14-day-old worms. The cuticle is covered with spines which become minute at the posterior end of the body. Remnants of eyespots are present. The mouth is terminal or subterminal. The prepharynx usually is absent although a very short one was seen in extended specimens. The pharynx is elongate. The esophagus is well developed. The intestinal ceca terminate near the posterior margin of the testis. The testis is relatively large occupying much of the posterior fourth of the body. The oval ovary is immediately anterior to the testis and usually a little to the right of the mid-line. Near the ovary is a seminal receptacle of variable size. The vitelline follicles are scattered from the posterior margin of the gonotyl to near the posterior end of the body. The gonotyl is pyriform and 3-lobed with 2 sclerotized hooks as shown by Kuntz and Chandler (1956).

It usually is to the right of the mid-line. There is an oval seminal vesicle immediately posterior to the gut bifurcation. The eggs are elongate and operculate.

Measurements of 20 stained and mounted, 10- to 14-day-old specimens are: body length 0.27–.46, av. 0.36; body width 0.12–.21, av. 0.16; oral sucker length 0.031–.047, av. 0.037; oral sucker width 0.037–.047, av. 0.041; pharynx length 0.025–.034, av, 0.03; pharynx width 0.012–.022, av. 0.018; esophagus length 0.025–.05, av, 0.039; testis length 0.05–.093, av. 0.067; testis width 0.05–.078, av. 0.059; gonotyl approximately 0.042 long and 0.027 wide; ovary length 0.025–.05, av. 0.039; ovary width 0.019–0.047, av. 0.029; egg length 0.025–.031, av. 0.027; egg width 0.012–.016, av. 0.014.

One of 2 night herons, *Nycticorax nycticorax*, examined had many specimens of *H. yokogawai* in its small intestine.

Although Katsuta (1932a) stated that the mullet was the second intermediate host, no mullet were found to harbor this parasite in the present study. The Chinese catfish, *Clarias fuscus*, is found in some taro patch ditches and probably serves as a natural second intermediate host. Africa (1938) stated that *Haplorchis yokogawai* was the most frequently encountered haplorchid in man and the most frequently associated with cardiac failure. This species, therefore, constitutes a health hazard in Hawaii.

SUMMARY

The heterophyid trematodes: *Centrocestus formosanus* (Nishigori, 1924), *Stellantchasmus falcatus* Onji and Nishio, 1924, *Haplorchis taichui* (Nishigori, 1924) and *H. yokogawai* (Katsuta, 1932) have been found in Hawaii. *C. formosanus*, *H. yokogawai* and *S. falcatus* develop in the digestive gland of the fresh water snail, *Stenomelania newcombi* Lea collected in taro patch ditches of Oahu. *H. taichui* and *S. falcatus* develop in the digestive gland of the snail, *Tarebia granifera* Lam. collected in the same ditches. *C. formosanus* uses *Gambusia affinis* and *Xiphophorus helleri* as natural second intermediate hosts. Occasionally one to a few metacercariae were found in the mullet, *Mugil cephalus*. Adult *C. formosanus* have been found in the night heron, *Nycticorax nycticorax*, and in rats. Experimental infections were obtained in rats and cats. *Stellantchasmus falcatus* metacercariae were found in all mullet, *Mugil cephalus*, collected in Hawaii. Experimental infections were established in mullet collected in Baja California. Experimental infections of adult worms were established in cats. Natural infections of *Haplorchis taichui* were found in *Gambusia affinis*, *Mollienesia formosus* and, rarely, *Mugil cephalus*. Experimental infections were established also in the gold fish, *Carassius auratus*. Cats served as experimental definitive hosts. A natural definitive host is the night heron, *Nycticorax nycticorax*. *Haplorchis yokogawai* metacercariae were experimentally established in the Chinese catfish, *Clarias fuscus*. Cats served as experimental definitive hosts and the night heron, *N. nycticorax*, as a natural one.

All of these species of Heterophyidae constitute a health hazard in Hawaii.

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EXPLANATION OF PLATES AND FIGURES

(All drawings made with the aid of a camera lucida)

Abbreviations

b—excretory bladder; c—cystogenous gland; e—eyespot; ex—expulsor; f—flame cell; fi—fin; g—genital primordium, gonotyl in adults; i—intestine; m—metacercaria; o—oral sucker; ov—ovary; p—pharynx; pg—penetration gland; pr—prostate cells; s—seminal vesicle; t—testis; u—uterus; v—vitellaria; vs—ventro-genital sac.

PLATE I

FIGURE 1. Portion of a sporocyst of *Centrocestus formosanus.*

FIGURE 2. Cercaria of *C. formosanus.*

FIGURE 3. Portion of gill filament of *Carassius auratus* containing six metacercariae of *C. formosanus.*

FIGURE 4. Young redia of *C. formosanus.*

PLATE II

FIGURE 5. Older redia of *C. formosanus.*

FIGURE 6. Redia of *Stellantchasmus falcatus.*

FIGURE 7. Outline of cercarial body and some details of tail of *S. falcatus.*

FIGURE 8. Some details of cercarial body of *S. falcatus.*

PLATE III

FIGURE 9. Mature *S. falcatus,* ventral view.

FIGURE 10. Young adult of *S. falcatus,* ventral view.

FIGURE 11. Gonotyl of *S. falcatus,* ventral view.

FIGURE 12. Terminal genitalia of *S. falcatus.* Not all of the prostate cells are shown.

FIGURE 13. Metacercaria of *S. falcatus.*

PLATE IV

FIGURE 14. Outline of usual shape of Heidenhain-fixed cercaria of *Haplorchis yokogawai,* lateral view.

FIGURE 15. Preoral spine arrangement of *H. yokogawai* cercaria.

FIGURE 16. *H. yokogawai* cercaria, dorsal view.
FIGURE 17. Preoral spine arrangement of *Haplorchis taichui* cercaria.
FIGURE 18. *H. taichui* cercaria, ventral view of body, dorsal view of tail.
FIGURE 19. Outline of usual shape of Heidenhain-fixed cercaria of *H. taichui*.

PLATE V

FIGURE 20. Redia of *H. yokogawai*.
FIGURE 21. Redia of *H. taichui*.
FIGURE 22. Adult *H. taichui*, experimental infection in a cat.
FIGURE 23. Gonotyl of *H. taichui*.
FIGURE 24. Young adult *H. yokogawai*, ventral view.

RESEARCH NOTE

MODIFICATION OF THE FORMALIN-ETHER FECAL CONCENTRATION TECHNIQUE FOR USE WITH SWINE FECES

The routine sugar flotation techniques as employed to detect coccidial oocysts were found to be unsuitable for levitating other protozoan cysts in swine manure. Therefore, the formalin-ether technique (Ritchie, 1948, Bull. U. S. Army Med. Dept. **8**: 326) normally used for human fecal examination was employed, and with certain modifications found to be satisfactory.

Briefly, the formalin-ether technique involves filtering a small sample of human feces through gauze, washing, and finally centrifuging with formalin and ether. The fecal residue thus obtained may be removed from the centrifuge tube with an applicator stick and mixed with either saline or iodine solution on a glass slide. However, since the content and consistency of swine fecal material differ from that of human beings, certain changes in Ritchie's technique had to be devised.

The initial preparations are the same as if a sugar flotation method is to be employed. First, 10 g of swine feces is thoroughly mixed with about 50 ml of water in a Waring Blendor for 2 minutes; this mixture is poured into an Erlenmeyer flask and enough water added to make a total volume of 300 ml; 15 ml is then poured, without filtering, directly into a graduated centrifuge tube and centrifuged at 1,800 r.p.m. for 2 minutes. If the supernatant fluid is very cloudy, a second washing may be carried out. The supernatant is poured off, and approximately 11–12 ml of 7.5% formalin is added to, and thoroughly mixed with, the sediment; about 3 ml of ether is then poured in, and the entire contents are well shaken, and then centrifuged at 1,500 r.p.m. for 2 minutes. The resulting layer of ether with trapped debris, and the formalin are poured off. Water may now be added up to 3 ml (or to any other desired level) and mixed with the sediment.

Using a Stoll pipette, 0.075 ml of the mixture is removed to a glass slide and covered with a 22-mm square cover glass. Counts of helminth eggs and protozoan cysts can then be made, if desired, and the approximate number per gram of feces calculated. Otherwise, a small drop of the mixture may be added to a drop of an iodine solution for easier identification of cysts. Similarly, a small portion of the sediment itself may be removed with a pipette and mixed with sufficient water on a slide to disperse the debris and make examination easier; in this case a larger cover glass may be required.

The above technique is of particular value in surveys to determine the presence of human protozoan parasites in swine. This technique has also been successfully used with fecal material from cattle and sheep.—ELLIOTT LESSER, *Regional Animal Disease Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Auburn, Alabama.*

PLATE I

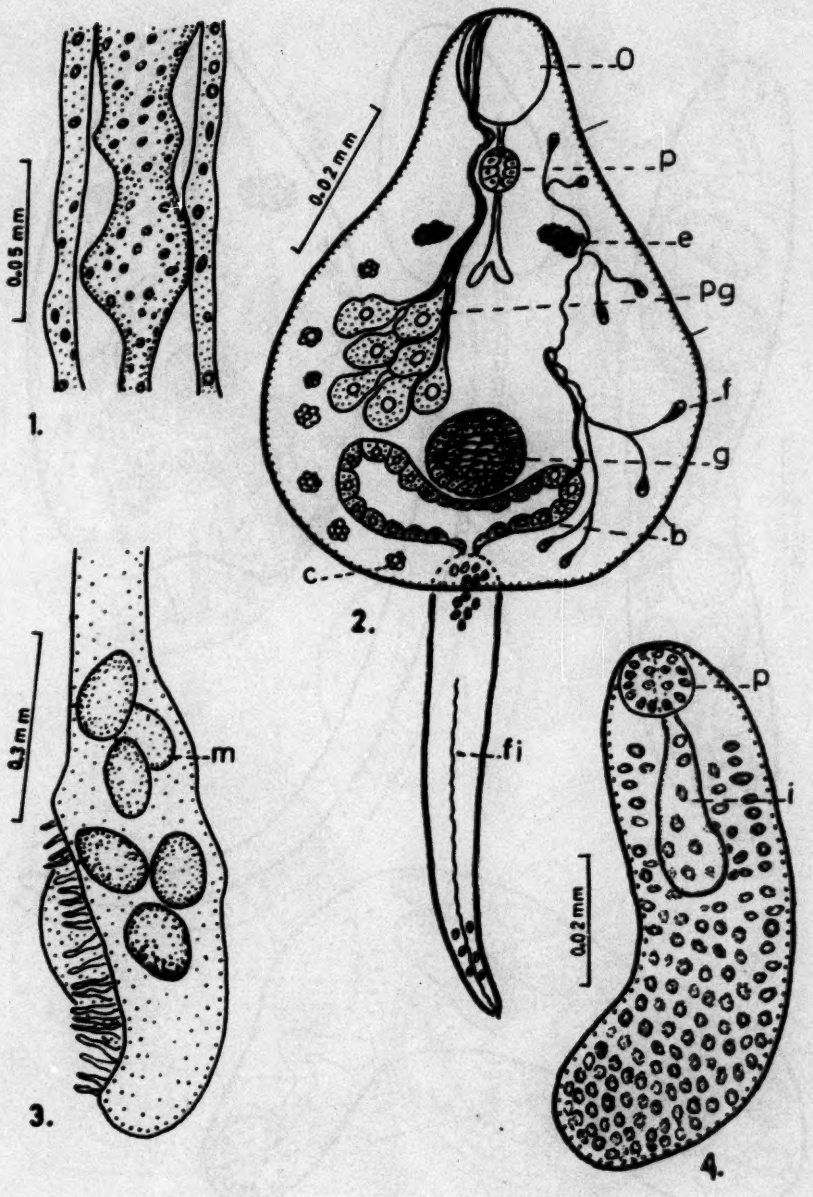


PLATE II

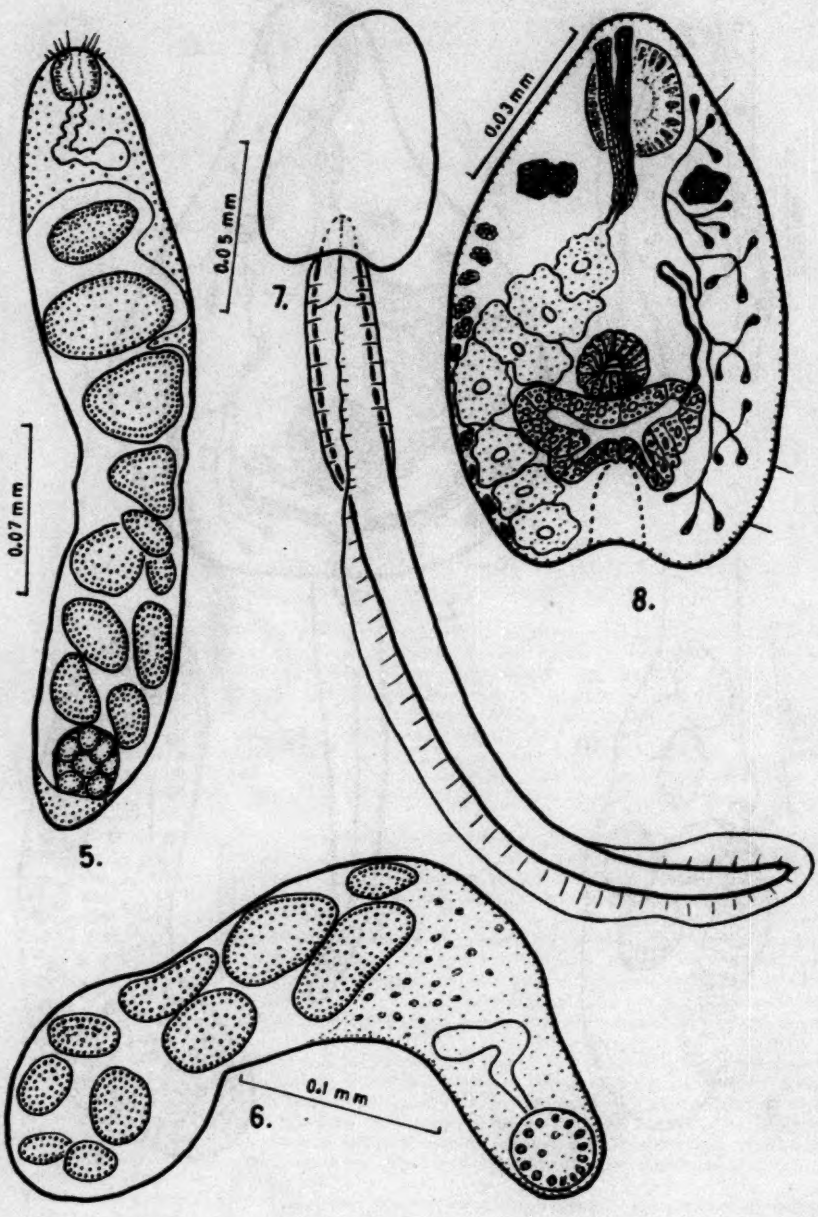


PLATE III

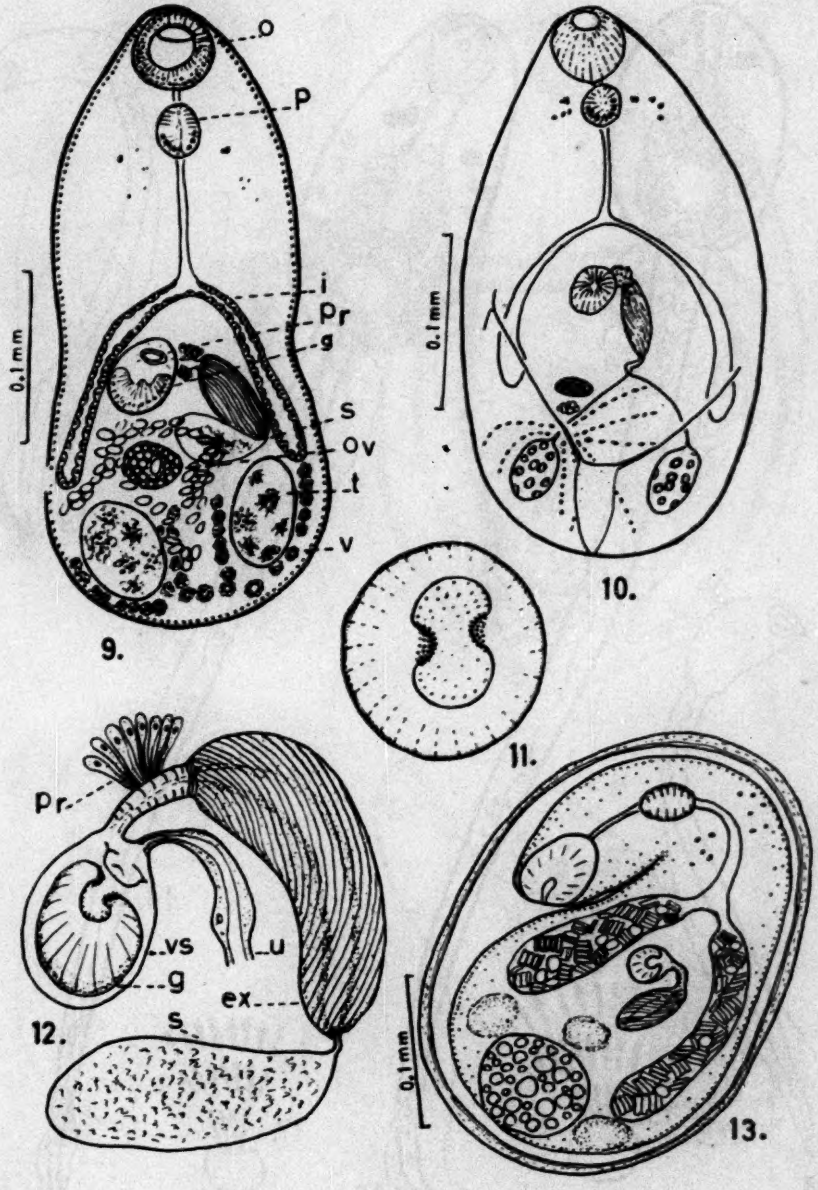


PLATE IV

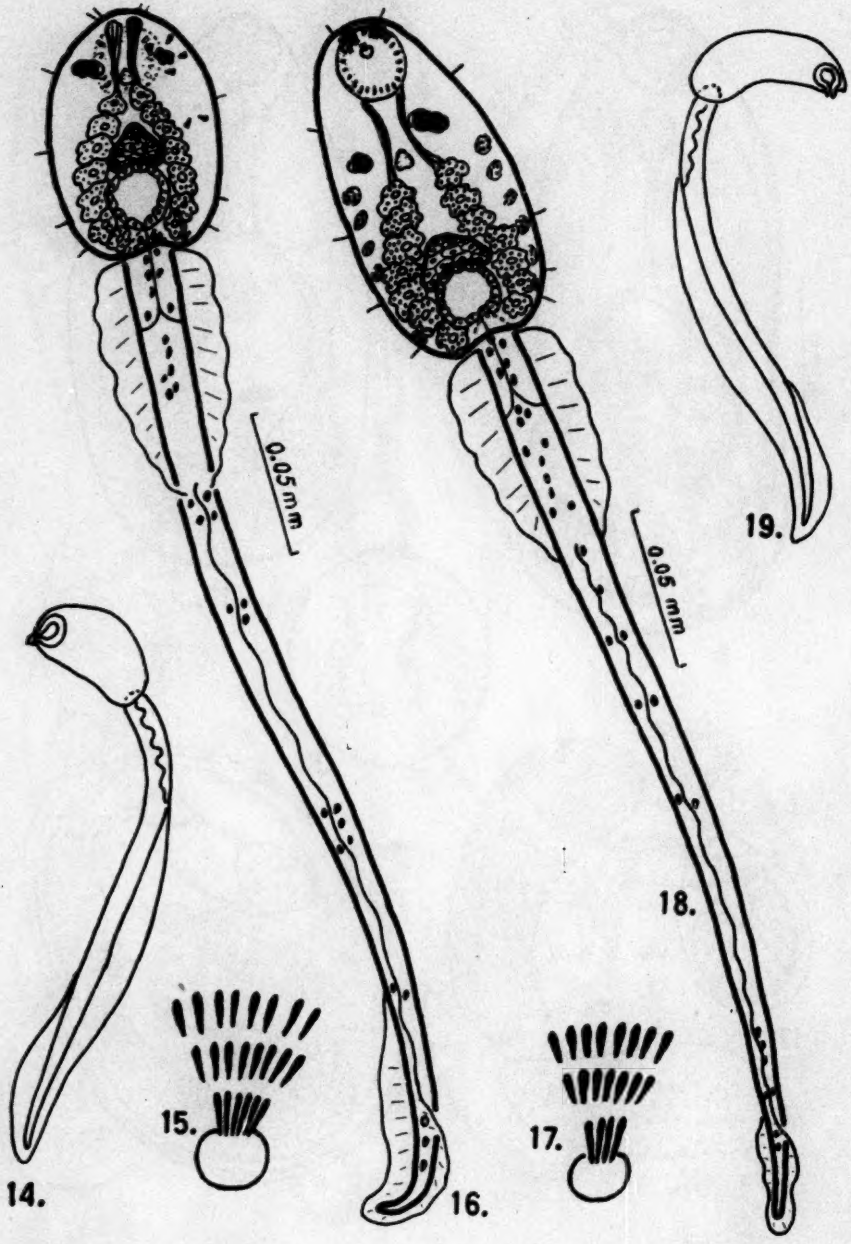
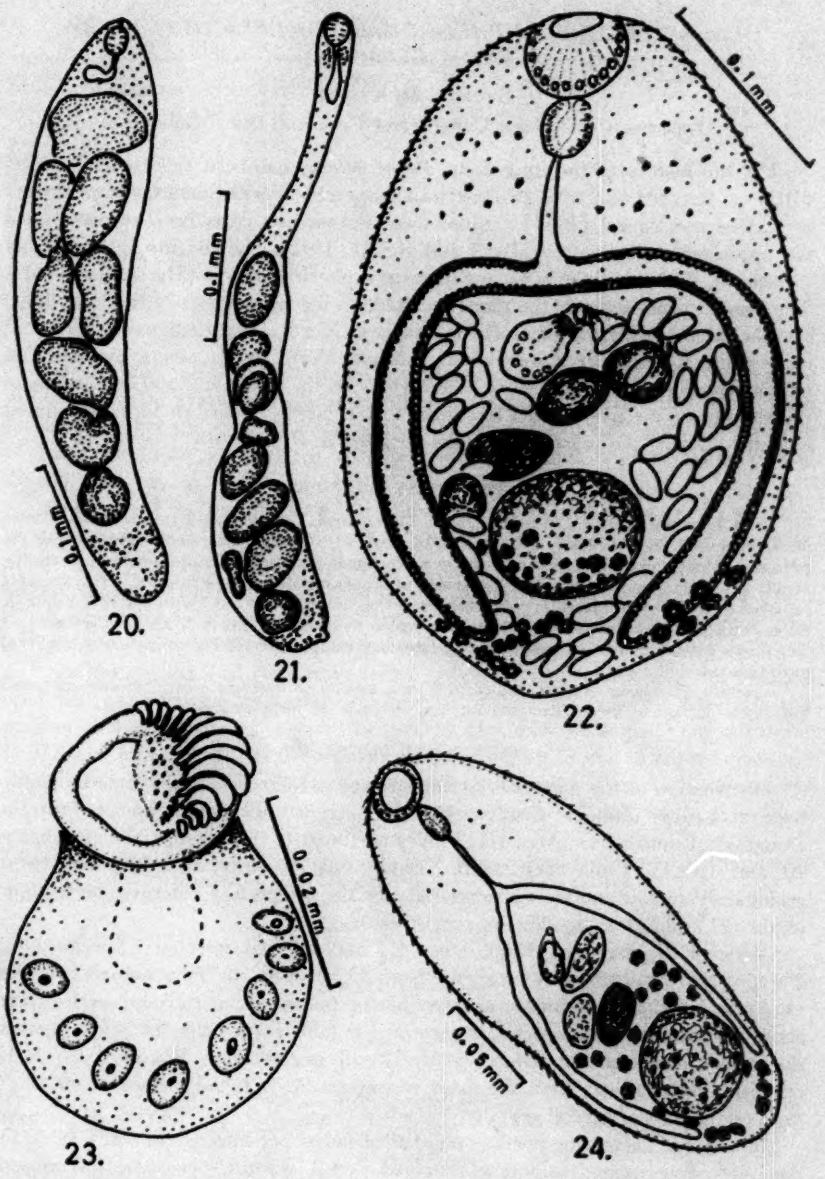


PLATE V



OBSERVATIONS ON *PARAGONIMUS KELLICOTTI* WARD FROM GEORGIA

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The life history of the lung fluke, *Paragonimus*, has been reported by Ameel (1934). Records of its North American hosts and distribution were summarized by LaRue and Ameel (1937). Since then, *Paragonimus* has been reported from the oppossum in Tennessee (Byrd and Reiber, 1942), the red fox in Minnesota (Erickson, 1944), and the house cat in eastern North Carolina (Hardcastle, 1941). The present paper extends the range and adds to the list of hosts of the lung fluke.

The taxonomy of the genus *Paragonimus* in North America remains unsettled; some workers recognize *Paragonimus kellicotti* Ward, while others consider this only a synonym of *P. westermani* (Kerbert). In the absence of any decision as to the validity of the name given by Ward to the North American form, specimens examined during the present study are assigned to *P. kellicotti*.

MATERIALS AND METHODS

From December 7, 1956 to March 12, 1957, 228 mammals representing 8 species were collected on 4 areas in Baker, Calhoun, and Dougherty counties, Georgia, and were examined for the presence of lung flukes. Gross examinations of the lungs were made for the presence of readily visible cysts; if no cysts were found, the animal was not considered to be infected. Worms were removed from the cysts, placed in cold water until they relaxed, and fixed with hot 10% formalin. Some were flattened with a microscope slide before fixation and others were fixed unflattened. The flukes were then washed, passed through standard dilutions of alcohol to 70%, and measured to the nearest millimeter.

Crayfish, *Cambarus sp.*, were collected from a swamp on one area where infected mammals had been found, and were examined for the presence of metacercariae.

RESULTS AND DISCUSSION

Distribution of the mammals examined was as follows: Area I, 5 miles southwest of Leary, Calhoun County, 46; Area II, 1 mile southwest of Pretoria, Dougherty County, 133; Area III, 3 miles northeast of Arlington, Calhoun County, 16; and Area IV, 1 mile northeast of Newton, Baker County, 33. All 4 areas were ecologically similar and were characterized by the presence of extensive bottomland hardwood swamps along slow-moving streams.

None of the mammals from Areas III and IV was infected. Specimens of *Paragonimus kellicotti* were recovered from 53, or 30%, of 179 mammals collected on Areas I and II. Incidence of infection for the opossum, raccoon, wildcat, and striped skunk, the 4 host species, is presented in table I; only the difference between the striped skunk and opossum was statistically significant. Six, or 35%, of 17 crayfish, *Cambarus* sp., from the main swamp on Area I had metacercariae of *P. kellicotti* in the pericardial region.

In order of decreasing average number of flukes per infected animal (Table I), the hosts were ranged as follows: striped skunk, wildcat, opossum, and racoon. All the differences were statistically significant except that between the wildcat and striped skunk.

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TABLE I. *Incidence of* Paragonimus kellicotti *in 179 mammals from 4 areas in Baker, Calhoun, and Dougherty Counties, Georgia*

Species	Area	Number examined	Number positive	Percentage positive ± S.E.	Number of flukes per infected host	
					Range	Mean ± S.E.
Opossum, *Didelphis marsupialis*	I + II	90	39	43 ± 5.2	1–23	7.4 ± .42
	III + IV	6	0	— —	—	— —
Cottontail rabbit, *Sylvilagus floridanus*	I + II	8	0	— —	—	— —
	III + IV	6	0	— —	—	— —
Red fox, *Vulpes fulva*	III + IV	2	0	— —	—	— —
Gray fox, *Urocyon cinereoargenteus*	I + II	2	0	— —	—	— —
	III + IV	3	0	— —	—	— —
Raccoon, *Procyon lotor*	I + II	32	7	22 ± 7.3	1–3	2.1 ± .47
	III + IV	14	0	— —	—	— —
Striped skunk, *Mephitis mephitis*	I + II	41	6	15 ± 5.6	2–25	11.7 ± .32
	III + IV	15	0	— —	—	— —
Wildcat, *Felis rufa*	I + II	6	1	17 ± 15.3	11	11.0 ± .00
House cat, *Felis domestica*	III + IV	3	0	— —	—	— —

Size parameters of mature flukes are presented in Table II. An analysis of differences in the average length of unflattened speciments revealed that flukes from the 4 hosts differ significantly from each other. In order of decreasing average size of flukes, the hosts were ranged as follows: striped skunk, wildcat, opossum, and racoon.

As indicated in the foregoing, the same order prevailed when the hosts were ranged according to decreasing average size of flukes and to decreasing average number of flukes per infected animal; that is, the species of host which had the largest number of flukes also had flukes of the largest average size, and there was a direct correlation between these 2 statistics for the 4 hosts. However, flukes from animals of the same species did not manifest such correlation. This was true for all 3 of the hosts which had 6 or more individuals infected. The number of infected opossums was adequate for a statistical analysis of the relation between the size and number of flukes. In this species, the number of flukes per animal ranged from 1 to 23; the average length of these flukes ranged from 10.75 mm in animals infected with 13 individuals, to 8.37 mm in those with 20 individuals. Flukes from opossums infected with 1 to 5 individuals had an average length of 9.67 mm, while those from

TABLE II. *Comparison of size parameters of adult* Paragonimus kellicotti *from 4 species of hosts from Dougherty and Calhoun Counties, Georgia*

Number examined*	Characteristic	Unflattened			Flattened	
		Length mm	Width mm	Thickness mm	Length mm	Width mm
		Didelphis marsupialis				
42–80	Mean ± SE.	10.4 ± 0.21	3.9 ± 0.11	2.3 ± 0.08	9.4 ± 0.15	3.8 ± 0.19
	Range	7–13	3–5	1–4	5–12	2–5
		Procyon lotor				
3–2	Mean ± S.E.	7.3 ± 0.33	3.3 ± 0.33	2.0 ± 0.00	6.5 ± 1.50	4.0 ± 1.0
	Range	7–8	3–4	2–2	5–8	3–5
		Mephitis mephitis				
24–33	Mean ± S.E.	15.7 ± 0.29	6.7 ± 0.13	3.9 ± 0.13	14.6 ± 0.26	6.2 ± 0.18
	Range	13–19	5–8	3–5	12–19	5–8
		Felis rufa				
5–6	Mean ± S.E.	12.4 ± 0.24	5.4 ± 0.24	3.6 ± 0.24	11.2 ± 0.98	5.5 ± 0.22
	Range	12–13	5–6	3–4	10–12	5–6

* Number to left of dash indicates the number of unflattened specimens; number to right of dash indicates the number of flattened specimens.

animals infected with 10 to 20 individuals averaged 9.94 mm; the difference was not significant. It is believed, therefore, that the host species infected with flukes of the largest size and in largest numbers has a physiology which provides the parasite with an optimal environment. Thus, a combination of average number and average size of lung flukes from a given host species constitutes a relative measure of host susceptibility when flukes from various hosts are compared.

LaRue and Ameel (1937) listed a number of mammalian hosts of *Paragonimus* but agreed with Wallace (1931) in considering the mink to be the normal definitive host in North America. Ameel found the parasite in 17% of 563 mink (1931) and in 12% of 79 muskrats (1932a); he thought that under certain conditions the muskrat might serve as the definitive host as well as the mink.

In comparison of incidence of infection by lung flukes, both the food habits and relative susceptibility of the hosts also should be considered. Of the mammals found to be hosts by the present study, the racoon probably eats the most crayfish and is, therefore, most frequently exposed to infection. It is also the least susceptible of the hosts; of 15 flukes recovered from 7 racoons, all were small and 6 appeared to be degenerative. Cysts from which these flukes were recovered were much larger than those in other hosts and were filled with either dark fluid or granular material. Large cysts which contained only granular material were found in some of the infected and 5 noninfected racoons. In external appearance, these cysts were typical of those which contained flukes. These observations on the low susceptibility of the racoon are supported by Ameel's (1931) unsuccessful attempt to infect one experimentally.

The high prevalence of infection in the opossum is probably a reflection of both its susceptibility and its habit of feeding extensively on crayfish. Forty-three per cent of the opossums were infected; this is more than double the rate of 17% reported for mink by Ameel (1931). However, the figures are not strictly comparable, for the mink were collected from scattered localities in Michigan and northern Ohio, and the opossums were from 2 restricted areas.

It seems unlikely that wildcats feed on crayfish to any appreciable extent. However, Ameel (1932b) has shown that lung flukes from 1.0 to 1.5 mm long remain in the pleural and abdominal cavities of white rats for months and that these worms will infect the domestic cat. In a similar manner, the wildcat could become infected by eating wild rodents which had eaten infected crayfish.

The striped skunk, the most susceptible host in the present study, had the lowest rate of infection, probably because skunks usually do not frequent areas where crayfish are abundant. The striped skunk and raccoon have not been reported previously as hosts of *Paragonimus kellicotti*.

SUMMARY

The lungs of 228 mammals, collected from 4 ecologically similar areas in Baker, Calhoun, and Dougherty counties, Georgia, were examined for *Paragonimus kellicotti*. No flukes were found in mammals from 2 of the areas, but 30% of 179 mammals from 2 areas in Calhoun and Dougherty counties were infected. Incidences of infection were as follows: opossum 43%, raccoon 22%, wildcat 17%, and striped skunk 15%. The striped skunk and raccoon have not been reported previously as hosts of *Paragonimus kellicotti*. The cottontail rabbit, gray fox, red fox, and domestic cat were negative.

The species of host which had the largest average number of flukes also had flukes of the largest average size, and there was a direct correlation between these 2 statistics for the 4 hosts. A combination of the 2 statistics may constitute a relative measure of host susceptibility. In order of decreasing relative susceptibility, the hosts were: striped skunk, wildcat, opossum, and raccoon.

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DEMODEX CRICETI N. SP. (ACARINA: DEMODICIDAE) WITH NOTES ON ITS BIOLOGY

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Department of Zoology, University of Massachusetts, Amherst, Massachusetts

Demodex criceti n. sp. was discovered in the course of the senior author's work at Cornell University. A provisional description of this species was made at that time (Nutting, 1950), but further study was needed to establish its identity as a distinct species. The prosecution of this work revealed several interesting features of the biology of the mite which are included below along with the species description.

Demodex criceti n. sp.
(all measurements are in microns)

Description (including characters of the genus): *Demodex criceti* is a diminutive member of the genus, the largest specimen (a female) ever found measuring 108.8. Unlike many members of the genus, it has a broadly rounded opisthosoma in all stages. *Male:* Mean body length, for 20 specimens, 87.4, mean opisthosomal length 30.5, podosoma approximately ½ total length (Fig. 1). Means with standard deviations for measurements of 20 specimens are given in Table I.

Capitulum (gnathosoma) markedly wider than long. Capitular spines small, basal peduncle slightly less than 3 and distal segment 4, this last sharply curved along its anterior margin and ends in a blunt point directed mediad (Fig. 4). A large thin seta measuring approximately 4 in length extends laterally from antero-lateral margin of palp (Fig. 1); 4 other short triangular setae found clumped on the inner anterior face of each palp.

Legs evenly spaced along podosoma with last pair barely visible from dorsal aspect. Genital orifice and anterior pair of dorsal tubercles lie directly over groove between first and second pair of legs (Fig. 3). This orifice elliptical, without operculum. Penis long (16), slender (1) and gently curved dorso-ventrally; its tip sharp and apparently sheathless while base moderately swollen. Dorsal podosoma free of markings except for 6 to 8 evenly spaced transverse striations directly above the last pair of legs.

Posterior epimeral plates open behind (Fig. 1). *Female:* Mean body length, for 20 specimens, 103.4. The greater length of the female is attributable solely to the longer opisthosoma. Mean opisthosomal lengths are 46.1 for the female, in contrast to 30.5 for the male. Other statistics based on measurements of 20 females are given in Table I.

TABLE I.—*Means and standard deviations based on measurements (in microns) of 20 specimens of each sex of* Demodex criceti

		Male	*Female*
Capitulum (gnathosoma)	length	14.7 ± 1.1	15.4 ± 0.9
	width	17.0 ± 1.1	19.8 ± 1.7
Podosoma	length	42.2 ± 1.8	41.6 ± 1.9
	width	31.6 ± 1.2	33.3 ± 1.3
Opisthosoma	length	30.5 ± 3.8	46.1 ± 2.9
	width	32.2 ± 0.6	33.6 ± 0.6
Total length		87.4 ± 4	103.4 ± 2.5

Capitular spines, legs, palpal setae, and podosomal striations identical to those in male.

Vulva lies directly behind short central groove between posterior epimera. It measures 8 × 1.5 with long axis corresponding to that of animal. Posterior wall of vulva lies only slightly behind a line connecting posterior margins of last pair of legs (Fig. 2).

As in the male the posterior sutures of the last pair of epimera are wanting.

Ova thin-shelled and transparent. A pigment mass marks future posterior of animal. Means with standard deviations for measurements of 20 ova are: Length, 37.9 ± 2.5; width, 20.2 ± 1.7.

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Larva: Very small larvae have no epidermal scales; the 6 "legs" are merely plates with 2 inconspicuous tubercles on each; the mouthparts are well developed. Later 4 epidermal scales are found, these with entire margins and a basal plate. Still later larvae possess 6 epidermal scales, the anterior pair without a basal plate (Fig. 6). The legs in the 2 later stages appear to be 2-segmented with 2 terminal claws.

Measurements of 20 larvae gave the following means and standard deviations: Length, 55.9 ± 2.5; width, 28.8 ± 3.3.

Nymph: In most cases it was difficult to separate protonymphs from deutonymphs. The only certain difference seems to be the presence in the latter of a well developed pair of anterior epidermal scales with basal plates opposite the first pair of legs (Fig. 5).

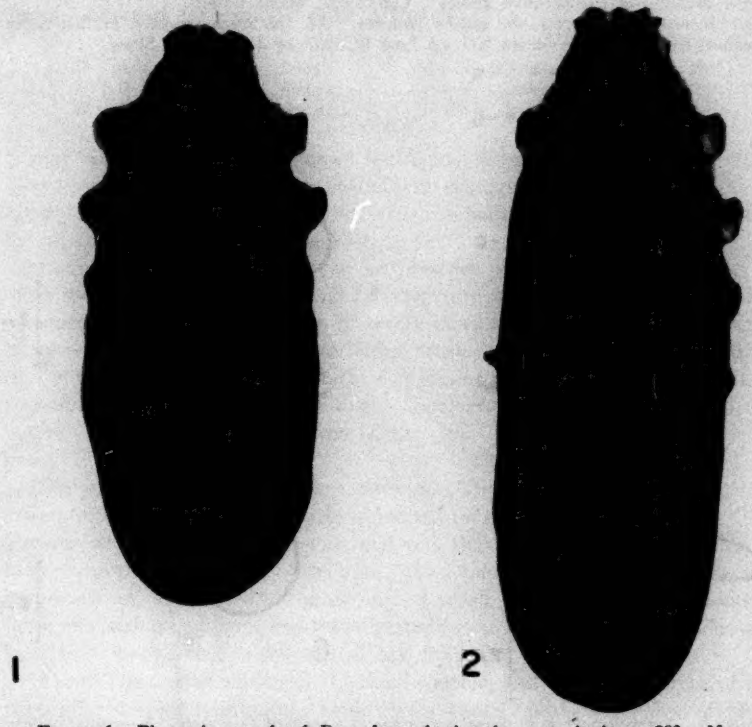

FIGURE 1. Photomicrograph of *Demodex criceti* male, ventral view ×900. Note lateral palpal setae and posterior epimera open behind.

FIGURE 2. Photomicrograph of *D. criceti* female, ventral view ×900. Lateral palpal setae, vulva and posterior epimera open behind.

Early protonymphs almost identical to early larvae except for presence of 4 pairs of plate "legs" and 6 pairs of epidermal scales. The two claws on each leg plate large and trifid. Later protonymphs have simple epidermal scales opposite first pair of legs. Legs in still further developed protonymphs and in deutonymphs 2-segmented, stubby and armed with 2 terminal claws.

Measurements of twenty nymphs gave the following means and standard deviations: Length, 84.4 ± 10.7; width, 30.5 ± 1.7. On 2 occasions deutonymphs were found with adult female mites developing inside. Each of these measured 105.6×35.2.

Diagnosis: Demodex criceti most closely resembles *D. muscardini* Hirst. It differs from this species in the following: 1) Smaller size of the adults of *D. criceti.* Hirst (1919) records these measurements for *D. muscardini:* Female, 173; male, 125–132. 2) Presence of large antero-lateral setae in the adults of *D. criceti;* in *D. muscardini* all setae are the same size and are clumped together on the anterior surface of the palp. 3) Male orifice opens in line with anterior tubercles and above the transverse groove between the first and second pair of legs in

D. criceti whereas the male orifice opens slightly anterior to the posterior tubercles and above the groove between the second and third pairs of legs in *D. muscardini*. 4) In the nymphs of *D. criceti* the epidermal scales are not toothed while 4 to 6 teeth (denticles) are present on the outer margins of the epidermal scales of *D. muscardini*.

D. criceti also bears some resemblance to *D. soricinus* Hirst, *D. ermineae* Hirst and *D. arvicolae* var. *apodemi* Hirst. However, these 3 species possess operculae in the males and have capitular spines which differ markedly from *D. criceti* (see Hirst 1917, 1918, 1919, and 1921).

Types: Slide #29 in the senior author's collection contains the following specimens designated as types: a male (ringed in black) as holotype, a female (ringed in white) as allotype, 3 other males and 5 females as paratypes. Another slide with 9 paratype specimens will be sent to the Museum of Comparative Zoology, Cambridge, Massachusetts.

Host: Mesocricetus auratus, the golden hamster. All specimens reported in this paper were obtained from laboratory strain N-1, on June 19, 1957, by Dr. Miriam Shaw.

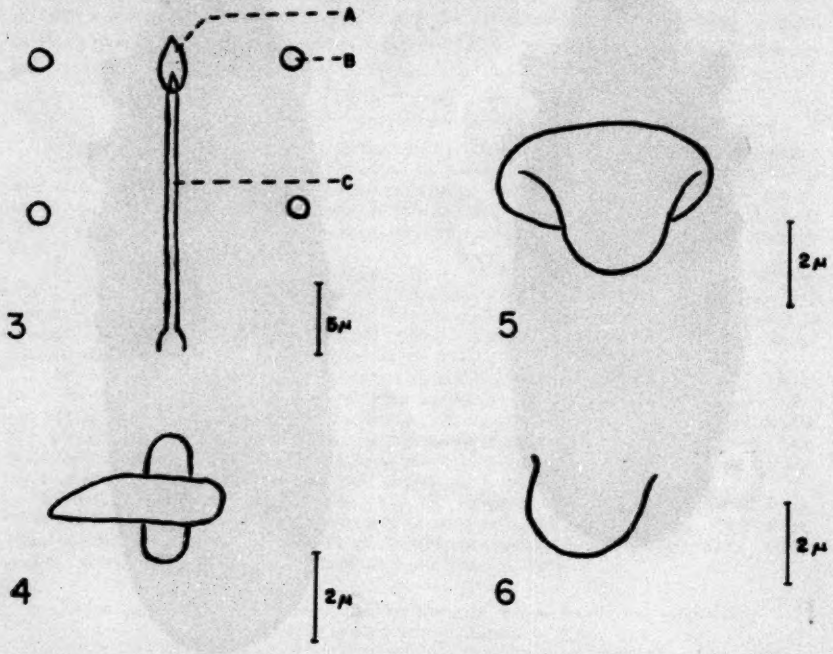

FIGURE 3. Anterior portion of dorsal podosoma of a male of *D. criceti*. A—genital orifice; B—dorsal tubercle; C—penis.

FIGURE 4. Capitular spine of *D. criceti*.

FIGURE 5. Nymphal epidermal scale with basal plate of *D. criceti*.

FIGURE 6. Larval and anterior nymphal epidermal scale of *D. criceti*.

DISCUSSION

Hirst (1919) admirably recounts the difficulties involved in assigning true species status to members of the genus *Demodex*. *D. criceti* is the first species in which statistically significant numbers of all stages of the life cycle have been measured. It seems that even here the large standard deviation reported in the nymphal stage indicates either that several molts occur, that a size difference related to sex may be involved or that both of these possibilities occur.

A further difficulty, under investigation in our laboratory, is the presence of another form of the same genus in the same host. Contrary to views expressed

previously (Nutting, 1950) it appears that elongate specimens of this genus in the golden hamster are distinct. Whether this difference is varietal or specific is to be determined. This is in line with a note by Hirst (1919) that *Demodex nanus* and *D. ratti* are distinct species even though found on the same host. In both cases ample opportunity should be present for crossbreeding (Wharton, 1957) and yet no intermediates have been found.

The relatively low standard deviations for all measurements of adults indicate that *D. criceti* is possibly must less variable in size than most members of this genus. Compare for example these extremes:

D. caprae	female	224–315 microns	(Cram 1925)
D. folliculorum	female	270–440 microns	(Hirst 1919)
D. criceti	female	96–108.8 microns	(This report)

It may well be that in many cases the highly divergent measurements represent mixed populations from the same host of both elongate and squat forms of *Demodex*. Hirst (1921) notes that for *D. gliricolens* the body varied from short to elongate for specimens found on the same host.

Pathology: Gross symptoms of the presence of *D. criceti* are wanting. Pigment spots often prominent in old hamsters are not associated with the presence or activity of the parasite. Even in severe cases no evidence of tissue reaction or pigmentation was found. Application of crushed mites in physiological salt solution to the mucous membranes of the eye produced no evident reaction. It would appear that unlike demodicids of dogs (Gmeiner, 1909), cattle (Armfield, 1923), horses (Bennison, 1943), and goats (Cram, 1925), *D. criceti* is a low grade pathogen.

Site of Infestation: Unlike any heretofore reported member of the genus, *D. criceti*, in all stages of its life cycle is located only in the host epidermis. The organisms are found singly in epidermal pits the dimensions of which are rarely larger than that of the body of the mite. As a rule these pits extend to the stratum germinativum, but rarely to the dermis. Larval, nymphal and adult mites lie in these pits with mouthparts and legs directed toward the host dermis. These mites have never been found in the normal hair follicle.

Food: The unique habitat of *D. criceti* coupled with the piercing type of mouthparts offer strong circumstantial evidence for a solution to the problem of demodectic food supply. Gruby (1846) thought that demodicids feed upon sebaceous gland secretions. Hirst (1919) stated that the food was no doubt liquid. Ayres and Anderson (1932) suggested that *D. folliculorum* is a scavenger of cellular debris. The fact that *D. criceti* is found only in pits which they obviously have cut in the epidermis would eliminate sebaceous secretions as a food element and point rather conclusively to epidermal cell contents as their food. Mites of the genus *Demodex* have been reported from such areas as the sebaceous glands in the goat (Cram, 1925), Meibomian glands in man (Chambers and Somerset, 1925) and sheep (Baker and Nutting, 1950), sweat glands in the dog (Hirst, 1919), and lymph glands in the dog (Canepa, 1941). It seems likely that most members of the genus *Demodex* use cell contents as their food.

Life Cycle: The number of stages in the life cycle of *D. criceti* conform closely to that reported by Hirst (1919) for *D. muscardini*. Since *D. criceti* has resisted

all attempts at culture the span of time in each stage and the number of molts per stage are not known.

The eggs are laid singly in the epidermal pit of the female. Sections of eggs both in the oviduct and presumably recently deposited show no sign of embryonic development (see report of viviparity in *D. folliculorum* by Fuss, 1937). A large mass of pigment marks the future posterior of the adult. Mouthparts appear in the end opposite the pigment spot prior to hatching.

The larval mouthparts are well developed and sections indicate that early in larval life food is taken into the gut. The tubercles on the basal plate are motile but are incapable of moving the animal. Even the stubby legs which take their place are incapable of pulling the animal across a flat surface. It seems probable that larval and nymphal legs and also epidermal scales are merely holdfasts which may serve to force the animal against new cell groups but do not propel the animal across the surface of the host.

Protonymphs, and rarely deutonymphs, have opisthosomae which are more truncated than rounded. The adult females discovered within the deutonymphal exoskeletons were active and in every external respect mature.

Both males and females are very active when removed from the host. No evidence of pairing as suggested by Chambers and Somerset (1925) for *D. folliculorum* has been found.

Mite Transference: Preliminary studies indicate that transference in *D. criceti* is from mother to young at the time of suckling. Examination of a limited number of pregnant female hamsters sacrificed at term showed no mites in the maternal blood or uterus or in the embryos. In 2 instances mites have been obtained from young at 5 days of age. Mites are quite often found in hamsters at 20 days of age. In over 100 instances young hamsters isolated at 20 days of age from isolated mothers were later found infested with *D. criceti.* Such a direct method of transfer has been suggested for *D. canis* by the careful work of Wernicke and Stolte (1923).

SUMMARY

1. A new species of mite, *Demodex criceti,* parasitic on the golden hamster, *Mesocricetus auratus,* is described.

2. The pathology of *Demodex criceti* indicates that it is a low grade pathogen.

3. Epidermal pits are reported as a new site of infestation for a member of the genus *Demodex.*

4. Circumstantial evidence is given which indicates that demodicids feed on cell contents.

5. A description is reported of the life cycle of *Demodex criceti.*

6. Preliminary studies are described which indicate direct transference of *Demodex criceti* from host mother to young at time of suckling.

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STUDIES ON LIGULA-INFECTED COMMON SHINERS (*NOTROPIS CORNUTUS FRONTALIS* AGASSIZ) IN THE ADIRONDACKS

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The life history of *Ligula intestinalis* Linnaeus never has been satisfactorily determined. There still is much to be learned about the development of eggs within the definitive host, the definitive host species, the time, place and manner in which the eggs are eventually deposited, and the time of hatching as well as the subsequent development of the newly hatched larvae up to and including the time of entry into the body cavity of the fish hosts. The explanations for many of these phenomena have been largely assumed, either on circumstantial evidence or through analogy with closely related worms.

Perhaps the foremost reason for the dearth of information on the life cycle of ligulas is the fact that the existence of adult worms is of such short duration in the digestive tract of avian hosts that the chance of finding them in casual collections is not especially great. According to Lagler (1948) and others the avian hosts of adult worms include "gulls and other water birds which eat infected fish." It may be inferred from these accounts that every fish-eating bird species is considered a potential host of ligulas. While this may be true the published data do not justify such assumption. Obviously this phase of the life history of the parasite cannot be solved completely and satisfactorily without sacrificing a great many birds, some of which are not especially common. Therefore we must rely on other means for furthering our knowledge of the parasite.

The writer had an opportunity to make observations and studies on the host-parasite relation of ligulas at Wolf Lake near Newcomb, New York, in the central Adirondacks from 1941 to 1943 in conjunction with his ichthyological investigations, and herein presents his data as a contribution to the life history of the species.

It was discovered that many heavily infected common shiners in this lake migrated shoreward throughout the summer and particularly during calm weather when they were not subjected to the damaging effects of wave action. Therefore the idea was suggested that observations on these shiners and on birds feeding in the shallow water would contribute data on the life history of ligulas.

The habits of parasitized common shiners were observed in shallow water on numerous occasions. They could be easily differentiated from other common shiners, or other minnows, by their markings and plumpness as well as by their actions. Those collected for analyses of parasites were taken with an ordinary $4' \times 6'$ minnow seine. Although the fish customarily swam near the bottom generally they were actually seen previous to capture. This method proved to be more efficient than "blind" seining.

Immense schools of common shiners on numerous occasions were observed in the sublittoral zone and some of these were obviously ligula-infected fish. The evidence seems to indicate that the shiners in this lake generally adhere to the gregarious mode of existence until they become cumbersome from the development of

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larval ligulas. Although a solitary existence often occurs once they leave the schools, the gregarious habit still persists, in part, since small groups of less than a dozen are sometimes encountered. Some infected shiners evaded capture by retreating to deeper water but most of them either assumed a course parallel with the shore or headed directly toward it. Their movements generally were very sluggish so that capture was easily accomplished. Even those that evaded the net by seeking deeper water generally returned to the shallow water immediately after danger was over.

Common shiners breed extensively in the outlet of Wolf Lake in late May or early June, but none of the fish observed on the spawning areas, from outward appearance, was infected with ligulas. As a matter of fact, very few ligula-infected

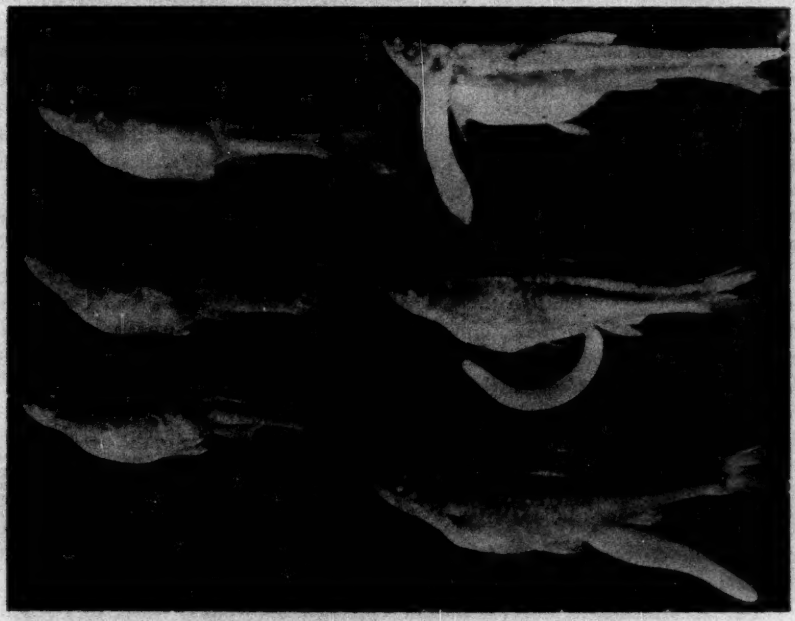

FIGURE 1. Typical ligula-infected common shiners from Wolf Lake showing worms escaping from the hosts.

common shiners of breeding age were encountered even in the shallow water of the lake at times.

Free-living ligulas were encountered in shallow water on numerous occasions and it is believed that many were liberated by rupturing the abdominal wall of the fish by excessive distention (Dence, 1940 and 1943). Some, apparently, had been liberated by predatory birds while feeding on their hosts. Others may have escaped through the vent or the thin abdominal wall tissues, inasmuch as they actually have been observed escaping in such manner when live hosts were placed in the killing solution or in a jar of water without provision for replenishing the depleted oxygen supply (Fig. 1).

In many localities common suckers are more susceptible to attack by ligulas

than any other fish species. This was not the case at Wolf Lake, however, as neither the indigenous common suckers nor the more abundant dwarf suckers were infected to a marked degree. Only 1 of the dwarf suckers that were opened contained ligulas and none of the other several thousand captured for study purposes and marking showed evidence of infection (Dence, 1948). The fact that dwarf suckers inhabit rather deep water throughout life, except for a brief period during the breeding season, may be significant. The parasite occasionally has been found in cutlips, *Exoglossum maxillingua* (Le Sueur), and in horned dace, *Semotilus atromaculatus* (Mitchill), at Wolf Lake and it probably also occurs in other species as well.

Hunter and Hunter (1931, p. 214) in their studies throughout the St. Lawrence River watershed of New York found only 4 species of fish infected with ligula. These were common suckers, *Catostomus commersonnii* Lacépède; dwarf suckers, *Catostomus commersonnii utawana* Mather; common shiners, *Notropis cornutus frontalis* Agassiz; and lake chub, *Couesius plumbeus plumbeus* Agassiz. They observed that "whenever infection with ligula occurred it was apt to be one of the dominant groups of parasites. Thus of the 34 common suckers and dwarf suckers examined from Lower Chateaugay Lake (no distinction being made in our records between these 2 forms) 15, or 44.1%, were afflicted by *Ligula intestinalis* larvae." These authors also speak of the "high degree of larval *Ligula* infection from suckers from Plumadore Pond, Ragged and Massawepie lakes" of the same watershed.

According to the records in the Roosevelt Station, ligulas occur in greater abundance in the Adirondacks than elsewhere in this State. Thus Van Cleave and Mueller (1932, p. 266) found only 8 worms of this species during the course of their intensive studies on the parasites of Oneida Lake fishes. By comparison this number is fewer than frequently occurs in a single common shiner at Wolf Lake.

Van Cleave and Mueller (1932) suggest the possibility of an association of the "larvae of this worm with the fish species living near the bottom in the open water." This was based on the fact that most of their ligula specimens were from small *Perca flavescens* (Mitchill) and *Boleosoma nigrum olmstedi* (Storer) taken with a beam trawl in deep water. The data from Wolf Lake indicate that bottom-feeding species from the deeper areas are less susceptible to attack than those species like the common shiner which feed at various levels and, frequently, near shore.

The fact that heavily infected shiners frequent shallow areas where they readily become accessible particularly to great blue herons and mergansers which have been observed foraging in such areas, indicates that infection takes place there rather than in deep water. Since the parasite matures very soon after entering the definitive host it may be logically assumed that ligula eggs are deposited in the water while the bird hosts are feeding. Schools of common shiner fry forage in the same shallow areas throughout the summer and it is quite possible that infection is accomplished during that period. The young of several other species of fish, likewise, migrate to shallow water but for some unexplainable reason are less susceptible to infection.

The horned dace, *Semotilus atromaculatus atromaculatus* (Mitchill), and cutlips, *Exoglossum maxillingua* (Le Seur) observed with ligulas could have been infected in shallow water, where they were captured, because they frequently inhabit such areas when foraging. The mere presence of species in shallow water

does not necessarily signify that infection will follow. Apparently the method and place of feeding or even the type of food are more significant.

Cooper (1918, p. 317) believes that "The production of eggs begins after about 36 hours, while the adults live for from 3 to 4 days only in the definitive hosts. Apart, however, from these and other closely related details . . . nothing is known, so far as the writer is aware, of the development of the oncosphere in the intermediate host up to the time when it becomes distinguishable as a small larva."

The definitive host species are not too well known due to the fact that relatively few adult worms are recovered from the digestive tracts of the bird hosts.

The potential avian enemies of common shiners at Wolf Lake include great blue herons (*Ardea herodias*), American mergansers (*Mergus merganser americanus*), herring gulls (*Larus argentatus smithsonianus*), common loons (*Gavia immer immer*), and kingfishers (*Megaceryle alcyon alcyon*). Although none of these birds were actually observed in the act of feeding on common shiners there is reason to believe that great blue herons and American mergansers, particularly the former, avail themselves of this abundant food resource. Both species forage in shallow water where infected shiners abound.

One or 2 pair of American mergansers, *Mergus merganser americanus*, and their respective broods of young usually are resident on Wolf Lake each summer. Most of their foraging expeditions are near shore and they frequently follow the contour of the lake for considerable distances. One or both adults, but sometimes only a female with her brood of young, have been flushed on numerous occcasions in shallow water where the infected comon shiners occur. Circumstantial evidence indicates that probably many shiners are eaten by these birds because these fish are better represented in shallow water than all the other species combined. The mergansers actually were observed feeding on a large group of minnows and sunfish.

Numerically, the herring gull, *Larus argentatus smithsonianus* Coues, is better represented at Wolf Lake than any of the other fish-eating birds, with 5 or 6 nesting pair. Furthermore, the resident population is augmented almost daily by visitors from other lakes. All of the gulls leave Wolf Lake immediately after the young of the year are capable of sustained flight in late August, and do not return until early spring.

Herring gulls are not considered to be an important factor in the common shiner-ligula relationship at Wolf Lake because they seemingly avoid those shallow areas where the ligula-infected minnows occur in abundance. Furthermore the pellets left by the gulls on the nesting rocks consist almost entirely of scales and bones of centrarchid fishes, particularly the red-bellied sunfish, *Lepomis auritus* (Linnaeus). While the gulls are frequently observed floating on the lake's surface within the red-bellied sunfish habitat they have never been observed in the act of feeding on living members of that or any other species. Eaton (1910, p. 130) believes that the principal food consists of dead, or disabled fish.

Normally one pair of adult common loons is resident on Wolf Lake each summer. These seldom forage in shoal water and particularly in habitats such as those utilized by the ligula-infected common shiners.

The American bittern and the kingfisher are casual visitors at the lake and were not observed feeding there.

Most of the ligula-infected common shiners were in the size class of 5 to 8 cm total length. However, there were a few as small as 4.6 cm and others as large as

10.5 cm. Only 122 specimens of assorted sizes from the several hundred collected were studied in detail for this report. Nearly all of the fish carried more than 1 worm—several as many as 7 or 8 and a few with greater numbers. While most of the worms were relatively small compared with ligulas of larger fish nevertheless the total weight or volume frequently amounted to more than 25%, and a few specimens as much as 50%, of the total weight or volume of the host.

The position of the worms varied somewhat but most of them were within the visceral cavity. They frequently coiled around the air bladder, intestine, and each other. A few had penetrated the pericardium, although this may have been the result of an attempt to escape after the hosts were placed in the preservative. Irrespective of the numbers generally all of the available space within the body cavity was utilized to the fullest extent.

While the parasitized shiners were not of breeding age the gonads normally would have been sufficiently developed to be easily recognized. This, however, was not the case and indicates that the worms created sterility. Other organs, likewise, showed evidence of degeneration.

SUMMARY

Although several species of minnows as well as 2 subspecies of suckers occur in Wolf Lake, larval ligulas were found almost exclusively in common shiners. Generally the weight (or volume) of the parasites represented over 25% and sometimes as much as 50% of the total weight of an individual fish. The gonads of infected fish were undeveloped while the other organs were somewhat degenerated and vastly distorted. The evidence indicates that American mergansers and great blue herons are the most likely definitive hosts.

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A STUDY OF THE SUMMER FILTH FLY POPULATION OF THE BERMUDA ISLANDS

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Studies conducted during the summer of 1955 on certain biting flies found in the Bermuda Islands (Williams, 1956a, 1956b, 1956c, Wirth and Williams, 1957, and Williams, 1957) disclosed that there existed at least 14 species which had not previously been reported. Six of these species were new to science and 8 were species known from other areas of the world (Africa, South America, Caribbean Islands, United States). It appeared advisable to ascertain what other species of potentially medically important arthropods might be found here and to this end a number of studies were made.

Flies have been known to be associated with a formidable list of pathogenic organisms and the addition of poliomyelitis virus to this list in recent years has increased interest in investigating the total fly populations of various communities.

Johnson (1913) studied the dipteran fauna of the Bermudas and Ogilvie (1928) published a list of Bermuda insects. In 1940 Waterston published a supplementary list of Bermuda insects.

PROCEDURE

Two fly traps (described by Williams, 1954) were utilized. One was placed behind a hardware store in the urban community of St. George in St. George's parish and the other in a rural area at Pampas Farms on the South shore in the parish of Smith's. Thus it was possible to contrast the filth fly population (flies attracted to decomposing organic matter for feeding or egg- or larva-deposition) of an urban area with that of a farm area in which there were cattle, horses, ducks, pigeons, chickens, dogs and cats as well as a marsh adjacent to the trapping area. Fish was used as a bait since it is generally accepted that it attracts more species of flies than other baits. The bait was changed every third day. The trapping period was from June 1, to August 23, 1957.

RESULTS

From Table I, it can be noted that some 12 species of flies were collected in each of the 2 traps, the same species being represented in each of the trapping localities. Of these 12 species the following had not been reported previously from the Bermudas: *Callitroga macellaria* (Fab.), the most common fly found, *Sarcophaga ventricosa* Wulp, *S. lhermipieri* (R–D), *S. rapax* Walk., *S. morionella* Ald., *Atherigona orientalis* Schin., and a species of *Euxesta* other than *abdominalis* L. reported by Ogilvie. *Sarcophaga assidua* Walk. was reported by Ogilvie but it is felt that this may have been *ventricosa* and that perhaps *S. helices* (Tows.) reported by the same author may have been *S. rapax* or *S. morionella*.

Although all species were taken in both trapping localities it will be noted from the table that *C. macellaria*, *Musca domestica* L., and *Phaenicia sericata* (Meig.) were captured more frequently in the urban area and that *Sarcophaga ventricosa*, *S. lhermipieri*, *S. morionella*, *Ophyra aenescens* Weid., *Synthesiomya nudiseta* (Wulp) and *Atherigona orientalis* were taken more frequently at the rural collect-

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TABLE I. *Results of filth fly catch, Bermuda Islands, June 1–Aug. 23, 1957*

Species	JUNE				JULY				AUGUST				Per cent of total fly catch represented by each species	
	St. George's		Pampas Farms		St. George's		Pampas Farms		St. George's		Pampas Farms		St. George's	Pampas Farms
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%		
Callitroga macellaria	1060	68.2	1072	44.1	2059	74.6	596	45.4	64	29.4	398	40.1	70.5	43.8
Musca domestica	169	10.9	276	11.5	304	11.0	36	2.7	24	11.0	23	2.3	11.0	7.1
Ophyra aenescens	142	9.1	142	5.9	88	3.2	171	13.0	6	2.8	130	13.1	5.3	9.4
Sarcophaga ventricosa	82	5.3	702	29.1	100	3.6	386	29.4	6	2.8	221	22.2	4.1	27.8
Phaenicia sericata	41	2.6	46	1.8	124	1.5	2	0.2	6	2.8	1	0.1	3.8	1.0
Sarcophaga rapax	37	2.4	30	1.2	14	0.5	6	0.5	1	0.5	11	1.1	3.2	3.3
Synthesiomya nudiseta	10	0.6	26	1.1	8	0.3	22	1.7	7	3.2	47	4.8	1.1	1.0
Fannia pusio	3	0.1	39	1.6	47	1.3	40	3.0	93	42.6	78	8.0	0.6	2.0
Sarcophaga thermipieri	8	0.5	53	2.2	8	0.3	36	2.7	1	0.5	47	4.8	0.4	2.9
Euxesta sp.	0	0.0	3	0.1	3	0.1	2	0.2	6	2.7	2	0.2	0.2	0.1
Atherigona orientalis	0	0.0	6	0.2	1	0.0	15	1.1	4	1.8	33	3.3	0.1	1.1
Sarcophaga morioncella	1	0.1	12	0.5	1	0.0	1	0.1	0	0.0	0	0.0	0.04	0.3
TOTAL	1553	99.8	2407	99.3	2757	99.4	1313	100.0	218	100.1	991	100.0	100.3	99.8

ing site. *Fannia pusio* Weid., *Sarcophaga rapax* and *Euxista* sp. were taken in about equal numbers at both collecting sites.

It was hoped that it would be possible to ascertain the population peak in time for each species but abnormal rains and winds during the study period prohibited these determinations. However, *Fannia pusio* and *Synthesiomya nudiseta* did appear to increase in numbers significantly in August over the previous 2 months (Table I).

The housefly is generally believed not to be attracted to any great extent to fish as a bait. This was found to be true by Williams (1954) when only 8 specimens were taken among some 18,118 flies (0.044%) in New York City and again in Michigan (Williams, 1956d) when only 44 were taken among some 17,146 flies (0.25%) trapped. However, in this study the housefly composed nearly 11% of the catch in St. George and 9.0% of the total catch, 832 in a sample of 9,239.

Of some 1,325 flies reared from 5 fish baits, 83% were *C. macellaria* and 17% *P. sericata*. No other species were recovered.

The total number of flies taken in the 2 trapping localities was not significantly different.

The overall filth fly population in the Bermuda Islands is very low. Undoubtedly among the main contributing factors to this condition are the frequent periods of high winds and driving rains.

SUMMARY

Flies attracted to decomposing fish were trapped in the town of St. George and at Pampas Farms in Smith's Parish, Bermuda Islands. The same 12 species were taken in each area but some were trapped in greater numbers in the urban habitat while others were caught more frequently in the rural habitat. The Bermuda Islands represents a new distribution record for *Callitroga macellaria, Sarcophaga ventricosa, S. lhermipieri, S. rapax, S. morionella, Atherigona orientalis* and an undetermined species of *Euxesta*. Fish, as a bait, seemed to be more attractive to the housefly, *Musca domestica*, in Bermuda than in parts of the United States where similar studies have been made. Only *C. macellaria* and *P. sericata* were reared from the bait.

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RESFARCH NOTE

THE HISTOTROPIC PHASE AND OTHER BIOLOGICAL ASPECTS OF *OSTERTAGIA OSTERTAGI*

In the past few years there have appeared in the literature reports on the histopathology and clinical manifestations in cattle due to *Ostertagia ostertagi* (Threlkeld and Johnson, 1948, Vet. Med. **43**: 446–452; Threlkeld and Bell, 1952, Auburn Vet. **8**: 137–140; and Bailey and Herlich, 1953, Auburn Vet. **9**: 105–110) and the morphogenesis of the free-living and parasitic developmental stages of this nematode (Threlkeld, 1946, Va. Agr. Exp. Sta., Tech Bull. **100**; and Douvres, 1956, J. Parasitol. **42**: 626–635). In all of the aforementioned publications, there is also included information concerned with the histotropic phase of this parasite in the abomasal mucosa.

Sommerville (1953, Nature **171**: 482; 1954, Austral. J. Agr. Res. **5**: 130–140) found that with *Ostertagia circumcincta* infections in sheep, the histotropic phase of this parasite is of variable length and that it is "associated with inhibited development and an extended prepatent period."

There is evidence in the foregoing publications of the present writer and those of others, that a correlation between the duration of the histotropic phase and developmental inhibition, such as reported by Sommerville (1953) for *O. circumcincta*, exists also for *O. ostertagi*. The present report, based on more detailed examination of some material studied in earlier investigations, and on new observations, attempts to show such a correlation and also brings forth other biological factors concerning this parasite.

The preserved specimens of 4-day larvae recovered from the mucosa and 5-, 7-, and 10-day-old "unattached larvae" retained from the 1946 study by Threlkeld were placed in lactophenol and re-examined at ×900 under both ordinary and phase illumination and compared with the descriptions and drawings of Douvres (1956). This was done to confirm the identity of the unattached larvae described by Threlkeld as *O. ostertagi* larvae. Other material utilized in this report consisted of 2 parasite-free yearling calves separately maintained during the experimental period in an elevated slat-floored pen. Calf I received repeated doses of infective *O. ostertagi* larvae at intervals of 2 to 3 weeks totaling 150,000 over a period of 7 months. This calf was slaughtered 10 days after the last dose of larvae. Calf II received 1 dose of 1,000 larvae 3 months before necropsy.

Additional material was collected during 1948–1954 from the examination of 27 abomasa removed from freshly slaughtered animals. The abomasa were fixed in 10% formalin, then dehydrated in alcohols. The excessive connective tissue was stripped from the organs and the mucosa and submucosa cleared in beechwood creosote. Worms were collected only from the mucosa by excising a small amount of tissue in which the worms were lodged. This tissue was tinted with azocarmine B and mounted in diaphane or permount. The various forms of *O. ostertagi* found are listed below.

Necropsy of calf I revealed developing fourth-stage larvae, young adults, and mature worms in the lumen of the abomasum. Serial sections from the muscularis mucosae show different stages of development of the parasite.

Examination of the mucosa of the abomasum of calf II which had received 1,000 larvae revealed 50 developing *O. ostertagi* forms. These were found in and on the mucosa and were comparable to 7- and 10-day-old larvae, and immature adults. Five mature worms were also found in the lumen.

In 27 animals necropsied, gravid adult worms were generally found in the pyloric regions of the abomasa in intimate contact with the mucosa but not in the mucosa. In only 4 cases were gravid adults found in the mucosa towards the cardiac region or in the fundus. These adult worms were found with their posterior portions anchored in the gastric pits. Some of

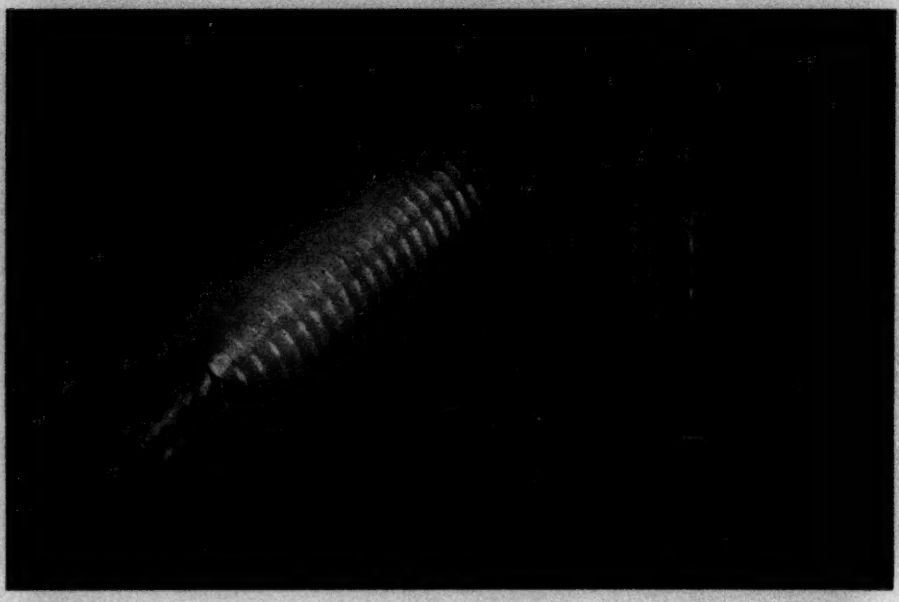
these females were in the act of ovipositing, and numerous eggs were in the pits around the vulva of the worms. Eggs were found deep in the mucosa of 8 animals. Some of these had reached the tadpole stage. One gravid female removed from the mucosa was found to be ovipositing in a tube, probably a straw, which was deeply embedded in the mucosa. The tails of young male and female worms, instead of the heads, were occasionally found protruding from the mucosa. Young adult worms in all stages of departure from the mucosa were observed in all abomasa. Some were found coiled near the surface and some were as far down as the muscularis mucosae.

This study discloses that the histotropic phase may be similar to that of *O. circumcincta*, Sommerville (1954). It may terminate at 96 hours, at 5 days, at 7 days, at 10 days, at 12-2/3 days, or at 15 days to 3 months post-infection.

Retardation in the growth of the parasite at various stages of development is noted in 2 calves, one of which received multiple doses of larvae and the other a small single dose of 1,000 larvae.

Necropsies of 27 beef yearlings show a high frequency of young male and female worms in the abomasal mucosa. They showed various stages of emergence.

It is suggested that since gravid females are rarely found in the mucosa younger females must escape in order to copulate. Some of these gravid females oviposit in the gastric pits.—W. L. Threlkeld, *Virginia Agricultural Experiment Station, Blacksburg, Virginia.*



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